IN THE UNITED STATES PATENT OFFICE

#12
10/3
JRP
5/4/01

Inventor : W. Roy KNOWLES, M.D.
Filing Date: 19 July 2000
Ser. No.: 09/619,142
Examiner: Vickie KIM
Art Unit: 1614

Honorable Commissioner of
Patents & Trademarks
Washington, DC 20231

APPEAL BRIEF

I. FORMALITIES

This Appeal Brief is submitted pursuant to the accompanying Notice of Appeal.

This is a Special Case subject to an approved petition to make special. The real party in interest is the applicant inventor. There are no related appeals nor interferences which will directly affect nor be directly affected by nor have a bearing on the Board's decision in this appeal.

Claims 1-22 stand twice rejected. There are no claim amendments.

A. Summary of Invention

1. Overview

The invention relates to maintaining healthy hair and preventing abnormal hair loss, by using *minoxidil* or a *testosterone blocker* (*e.g.*, progesterone) together with a skin *penetration enhancer*.

2. The Art and Its Shortcomings

Minoxidil in the systemic blood circulation is a potent anti-hypertensive cardiovascular drug. Minoxidil is also used topically as an anti-alopecia agent (*e.g.*, ROGAINE®). It has been understood that topical use with a skin penetrating agent would load the drug into the systemic blood circulation. This risks precipitating cardiac side effects. Such risk is unacceptable for cosmetic use for hair loss. Thus, minoxidil for hair loss has never included penetration enhancer. Specification at 2-7; Knowles, *Rule 132 Declaration*.

Progesterone is a birth control drug. Side effects include carcinogenicity, feminization, and impotency. Progesterone has been disclosed topically for hair loss. Such teaching, however, due to concerns over potential side effects, include a "blocking agent" and never include skin penetration enhancer. Id. at 7.

The foregoing is expressly taught by the references and is undisputed by the Examiner.

3. Dr. Knowles' Counter-Intuitive solution

Dr. Knowles has turned this conventional wisdom on its head. He has found that, contrary to the teachings of the art, penetration enhancer can safely be used with minoxidil or a testosterone blocker - **if** used properly. Specification at 8-9.

He tested his invention in rigorous, confidential clinical trials. His invention has been proven **ten times more effective** over prior art preparations, with **qualitatively better results**, with none of the **adverse side effects** feared in the prior art. Id. at 8, 12-14; Knowles, *Rule 131 Declaration;* Knowles, *Rule 132 Declaration.*

The claims are thus drawn to minoxidil or testosterone blocker combined with a specific amount of skin-penetration enhancer. Claim 1 reads:

> 1. A composition of matter intended for topical use in preventing or treating alopecia, or maintaining healthy hair, said composition of matter comprising:
>
> a) an active compound selected from the group consisting of: a pharmaceutically or cosmetically effective topical amount of a testosterone blocker and minoxidil, and
>
> b) a penetration enhancer, said penetration enhancer present *in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs.*

4. *Conclusion*

The references of record lack essential claim limitations. The references fail to enable (in fact actively teach away from) the claimed invention. The Applicant has "sworn behind" a key reference. Furthermore, the rejections must be reversed because the examiner refuses to produce an affidavit of references.

Thus, all pending rejections must be reversed.

B. Issues Presented

Whether Applicant has sworn behind Hoke?

Whether Hoke may be combined with Orentreich?

Whether any reference includes the claim limitation, "penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs"?

Whether any reference enables one to practice the claim limitation, "penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs"?

C. References

The Examiner relies on the following references:

1. *Hoke + Orentreich*

Orentreich, United States Letters Patent No. 5,053,403, teaches the topical use for hair loss of progesterone combined with a "blocking agent." Orentreich says progesterone has systemic side effects so serious that it should not be used for hair loss. Id. at col.1 lines 45-51 ("The serious side effects (such as decreased libido) produced by the systemic administration of antiandrogens precludes the systemic use of these drugs for the treatment of the above skin disorders. For example, progesterone is a highly active 5α reductase enzyme

inhibitor, but systematically disturbs the menstrual cycle in women").

Hoke, United States Letters Patent No. 5,994,319, similarly teaches progesterone's adverse systemic effects, col.4 lines 18-23. Hoke teaches that minoxidil has "potent" cardiovascular side effects and doesn't work well for hair loss. Id. at col.3 lines 4-14. Hoke advocates instead the use of his claimed antisense oligonucleotides (DNA) for hair loss. Id.

2. Bazzano + Mikulak

Bazzano, United States Letters Patent No. 5,183,817, teaches using retinoids for hair loss. Bazzano says that minoxidil alone *does not work*. Bazzano says,

> Minoxidil is recognized as being somewhat effective in producing new vellus hair growth and sparse terminal hair growth in a pre-selected group of subjects, However, its effect is far from satisfactory in most subjects. * * * [M]inoxidil may not be able to sustain the growth of terminal hairs from vellus hairs on the scalp. In the majority of subjects with alopecia, terminal hair growth on the scalp may not be initiated or sustained by the topical application of minoxidil nor by its systemic administration.

Id. at col.3, line 53-56 and col.4, lines 49-54. Bazzano teaches that systemic minoxidil presents serious cardiac side effect risks. Id. at col.3 line 49-52; col.43-45.

Mikulak, an abstract of 50(2) J. Pharm. Pharmacol. 153 (1998), discloses a new skin penetration agent (TPDS). The abstract compares it to other methods of drug administration.

Mikulak compares (1) TPDS; (2) a 50:50 propylene glycol ("PEG") alcohol vehicle (the control); (3) a "commonly used skin penetration enhancer"; and (4) oral administration ("We compared the TPDS with a 50:50 (vol./vol.) mixt. of propylene glycol and ethanol, a commonly used penetration enhancer, and with oral administration.").

3. Rajadhyaksha

Rajadhyaksha, United States Letters Patent No. 5,482,965, teaches improved skin penetration agents. Rajadhyaksha teaches that his compounds effectively deliver drugs into the systemic blood circulation. Id. at col.2 line 16-19. The compounds pass "through the skin and the systemic circulation" to the liver, and yield nontoxic metabolites. Id. "*Systemically active* agents are used in amounts calculated to achieve and maintain *therapeutic blood levels* in a human or other animal." Id. at col.3 line 53-60; col.10 line 11-14. Rajadhyaksha teaches using his compounds for systemic drug delivery. Id. at col.18 line 1-28. His compounds do in fact make drugs penetrate *completely though the skin*. Id. at col.18 line 55 to col.19 line 12.

D. Separately-Patentable Claims

The following groups of claims are separately patentable: 1, 2 and 3; 4, 5, 6 and 7; 8, 9 and 10; 11; 12, 13 and 14; 15, 16, 17 and 18; 19, 20 and 21; 22.

II. ARGUMENT

A. Hoke

Claims 1-22 stand rejected as obvious over Hoke in view of Orentreich. Hoke cannot render the claims obvious, because (1) the Applicant has sworn behind Hoke, (2) the art provides no suggestion to combine (and in fact teaches to not combine) these two references, (3) the references lack a claim limitation, and (4) the claimed invention shows secondary indicia of non-obviousness.

1. Applicant Antedates Hoke

A §103 rejection is overcome by swearing behind any one reference. MPEP §715.02 ¶4. Here, Applicant has sworn behind Hoke. *See* Knowles, *Rule 1.131 Declaration*. The Examiner accepted this Declaration without objection. *See Office Action* at 4-5 (14 March 2001). Thus, the rejection **must** be withdrawn. MPEP §715.02 ¶4.

2. Hoke Teaches Away From Using Penetration Enhancer with Progesterone or Minoxidil

Hoke teaches using nucleotides. Hoke says nucleotides are safe because they are "highly selective" genetic binders.

They thus do not pose the systemic side effect risk seen with minoxidil or progesterone. Orentreich teaches using progesterone together with "blocking agents."

Hoke and Orentreich teach away from combining penetration enhancer with minoxidil or testosterone blocker. Hoke says minoxidil is "a potent anti-hypertensive" cardiac drug. Hoke says that systemic use of minoxidil can risk cardiac arrhythmias. Hoke at col.5, line 4-6. Hoke thus teaches that minoxidil combined with a penetration enhancer may precipitate cardiac arrhythmias. Knowles, *Rule 132 Declaration* at ¶¶3-7, 14-17. Hoke also says minoxidil doesn't work. Hoke col.3, line 4-11 ("only 8% of patients reported a dense re-growth of scalp hair"). Thus, Hoke discourages using minoxidil *at all*, with or without penetration enhancer. Knowles, *Rule 132 Declaration*.

Hoke teaches progesterone's adverse side effects like "feminization or impotency." *Hoke* at col.4, line 18-24. Orentreich similarly teaches serious systemic side effects such as decreased libido and "systemic[] disrupt[ing] the menstrual cycle in women."

Orentreich concludes that cardiac arrhythmias, feminization and impotency are side effects generally **not acceptable** for treating hair loss. Orentreich says these side

effects **preclude systemic use** of these compounds for skin disorders. Orentreich at col.1, line 45-52.

Hoke and Orentreich thus teach that for hair loss, combining a penetration enhancer with minoxidil or progesterone is not worth the risk. They thus teach away from the claimed combination. Knowles, *Rule 132 Declaration*.

Significantly, the Examiner does not dispute this.

3. The Examiner's Rationale

The Examiner accepts the forgoing. What basis, then, does the Examiner offer to combine the references? The Examiner takes "official notice" that progesterone and anti-sense nucleotides have "the same biological pathway" and "work via same mechanism." Office Action at 3 (5 Oct. 2000). This assertion is baldly incorrect.[1]

Anti-sense nucleotides bind to sense nucleotides (genes) coding for 5α reductase, slowing production of it. Knowles, *Rule 132 Declaration*. In contrast, progesterone competitively binds to the 5α reductase receptor. The two types of compounds thus use completely different biological pathways;

---

[1] And improper. The suggestion to combine cannot be based solely on an officially noticed fact. *See* Ex parte Grochowski, No. 95-1343 at 5 (B.P.A.I. June 27, 1995). In re Eynde, 178 USPQ 470, 474 (C.C.P.A. 1973) elaborated, "The facts concerning the state of the art are normally subject to the possibility of rational disagreement among reasonable men and **are not amenable to the taking of [judicial] notice**. If evidence of the knowledge possessed by those skilled in the art is to be properly considered, it must be timely injected into the proceedings."

one affects gene translation **inside** the cell, the other affects receptors **outside** the cell. Id. One affects 5α reductase, the other affects a cell receptor. This is why Hoke himself says these two classes of compounds are *not* "interchangeable" and why Hoke's own compounds are patentably distinct from progesterone. Id.

Applicant asked for an affidavit of references to support the Examiner's incorrect assertion. The examiner refuses to respond. Because the Examiner refuses to respond, the rejection **must** be withdrawn. Ex parte Nouel, 158 USPQ 237, 239 (B.P.A.I. 1967) ("when the examiner judicially notices or to show wherein such matter, and such is challenged, *there is reversible error when the examiner fails to cite the well known thing on which he relies*").[2]

4. The Cited Combination Lacks An Essential Claim Element

The claims require "penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the

---

[2] In re Ahlert, 165 USPQ 418, 420 (C.C.P.A. 1970), explained some limits of official notice, commenting, "Assertions of technical facts in areas of esoteric technology **must always be supported** by citation to some reference work recognized as standard in the pertinent art. … Allegations concerning specific 'knowledge' of the prior art … should also be supported. … The facts so noticed serve to 'fill the gaps' … and **should not** comprise the principle evidence upon which rejection is based."

depth of hair bulbs." Here, the references lack this limitation. The Examiner **admits** this.

The Examiner, however, quips, "penetration enhancer … is not considered critical." Office Action at 5 (Oct. 24, 2000). The Examiner is not at liberty to simply ignore a claim limitation shown by the Specification and the Inventor's *Rule 132 Declaration* to be essential. Anticipation requires **all** limitations exist in the prior art. *E.g.*, Akzo N.V. v. U.S. Intern. Trade Comm'n., 808 F.2d 1471 (Fed.Cir. 1986), *cert. denied*, 482 U.S. 909.

The Examiner alternatively takes official notice combining penetrant and minoxidil "is common practice which has been utilized by the skilled artisan in the state of the art." Office Action at 5 (Oct. 24, 2000). Taking official notice that the claimed invention is known in the art is improper. In re Pardo & Landau, 214 USPQ 673, 677 (C.C.P.A. 1982) (official notice of level of skill in the art is reversible error); In re Spormann, 150 USPQ 449, 452 (C.C.P.A. 1966) (Board's official notice of "inherent" teachings of art is reversible error); 37 CFR §1.104(d)(2) (official notice "**must be supported**, when called for by the applicant, by an affidavit from the examiner"). Applicant thus asked for an affidavit of references showing such combinations in common practice and utilized by the

skilled artisan. Predictably, examiner refuses to respond. Thus, the rejection **must** be withdrawn. Nouel, *supra*.

5. The claimed invention shows secondary considerations of non-obviousness

The claimed invention shows many indicia of non-obviousness. The claimed combination solves a long felt need for a solution to a hairy, difficult problem. Dr. Knowles succeeded where others failed. Dr. Knowles' results were unexpectedly superior, showing *ten times* the effectiveness of topical minoxidil, with *qualitatively better* results. Knowles, *Rule 132 Declaration*. The Examiner accepted this secondary evidence without dispute. Thus, the invention should not be found obvious.

B. Bazzano

Claims 1-4, 8-10, 12-5 and 19-21 stand rejected as anticipated by Bazzano. Bazzano, however, fails to teach *every limitation* of the claims, and fails to *enable practicing* the claimed invention.

1. Bazzano Lacks Claim Limitations for All Disputed Claims

Bazzano does not teach skin penetrant. The Examiner admitted this, but quiped that this limitation "is not considered to be critical." Office Action at 5 (Oct. 24, 2000). The Examiner cannot simply ignore this claim limitation.

Because Bazzano does not disclose it, Bazzano cannot anticipate. *E.g.*, Akzo, *supra*.

Bazzano teaches minoxidil in a propylene glycol ("PEG")-ethanol vehicle.[3] This is not controversial; long before Bazzano, PEG-ethanol has been used as a cosmetically inert cosmetic vehicle, for ROGAINE® brand topical minoxidil and a variety of other cosmetics.[4] Pre-mixed PEG-ethanol is widely available commercially as an inert carrier vehicle (*e.g.*, The Neutrogena Company's VEHICLE/N™ carrier vehicles discussed in the Specification).

The Examiner, however, now says PEG-ethanol is "a commonly used penetration enhancer." Office Action at 3-4. **It is not.** The Examiner's own references say so.

Bazzano, at claim 24, calls PEG-ethanol an inert "vehicle." Knowles, *Supplemental Declaration*. Likewise, Mikulak teaches PEG-ethanol is an (ostensibly inert) vehicle used as the experimental control (Mikulak discloses a comparison of (1) TPDS; (2) 50:50 PEG-ethanol; (3) a "commonly used [albeit unidentified] skin penetration enhancer"; and (4) oral administration). Id.

---

[3] *See* Bazzano at Example I. The Examiner also says Example II teaches PEG-ethanol. Office Action at 3 (March 14, 2001). It does not.

[4] The Food & Drug Administration monograph on sunscreens treats PEG 5% as an inert vehicle. *See* 21 C.F.R. 352.70.

Further, assuming Mikulak's 50:50 PEG-ethanol aids skin penetration, Bazzano doesn't use it. Bazzano uses not a 50:50 mix, but a range which encompasses commercially-available ROGAINE®. Id. Minoxidil in a PEG-ethanol vehicle (*e.g.*, ROGAINE®) is only one tenth as effective as the claimed compounds, and cannot produce the qualitatively superior results of the claimed compounds. *See* Specification; Knowles, *Rule 132 Declaration*. This is undisputed.

Further, even assuming Bazzano's PEG-ethanol mix has skin penetration activity, Example I cannot anticipate as a matter of law. This is because claim terms are interpreted in light of the specification. Here, the Specification defines "penetration enhancers" (pg. 9-11) and "carrier vehicles" (pg. 17-20). PEG and ethanol, "alone or in combination," id. at 17, 18-19, are defined as "carrier vehicle," not "penetration enhancer." In other words, the claim term "penetration enhancer" was defined to exclude PEG-ethanol, as already known in the art in ROGAINE®. Thus, as a matter of law, Bazzano's PEG-ethanol cannot anticipate the claim term "penetration enhancer."

2. Bazzano Does not Enable The Claimed Invention

To anticipate, Bazzano must enable one of skill in the art to practice the claimed invention. Biogen Inc. v. Amgen

Inc., __ F.3d __, __ (D.Mass. 1999). Here, Claim 1 requires "penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs." It is undisputed that Bazzano does not enable this.

Further, it is undisputed that Bazzano actively teaches away from the claimed invention. Bazzano says that without added retinoid, *minoxidil just does not work*. *Supra* at §I.C.2; Knowles, *Rule 132 Declaration* at ¶11.

Because Bazzano lacks a claim limitation, and because it is undisputed that Bazzano does not enable (and in fact actively teaches away from) the claimed invention, the rejection must be withdrawn.

C. Rajadhyaksha

Claims 1-4, 8-10, 12 and 14 stand rejected as anticipated by Rajadhyaksha. Rajadhyaksha cannot anticipate the claims, because Rajadhyaksha lacks several claim limitations, and does not enable the claimed invention.

1. Rajadhyaksha

Rajadhyaksha teaches skin penetration agents. Rajadhyaksha teaches they deliver drugs into the systemic circulation. The reference teaches delivering drugs **completely**

through the skin into the systemic bloodstream. Id. at Example 32. Knowles, *Supplemental Declaration*. The reference says:

> Typically systemically active agents which may be delivered transdermally are therapeutic agents which are sufficiently potent such that they can be delivered *through the skin or other membranes to the bloodstream* in sufficient quantities to produce the desired therapeutic effect. In general this includes agents in all of the major therapeutic areas including … cardiovascular preparations including calcium channel blockers, beta-blockers, antiarryhthmics, antihypertensives, diuretics, vasodilators including general, coronary, peripheral and cerebral.

Id. at col.7 line 40-57.

The reference cannot anticipate the claimed invention, because the reference lacks various claim limitations and fails to enable the claimed invention.

2. Rajadhyaksha Lacks Essential Claim Limitations

Rajadhyaksha lacks various claim limitations. Claim 1 is limited to:

> b) a penetration enhancer, said penetration enhancer present *in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs*.

Rajadhyaksha does not teach delivering drugs "to the depth of the hair bulbs." To the contrary, Rajadhyaksha teaches delivering drugs **completely through the skin**, into the systemic bloodstream. Id. at Example 32. Rajadhyaksha teaches delivering drugs to the systemic blood stream using a 5%

concentration of 5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane ("A5A"). Id. at Examples 28-30. Rajadhyaksha teaches delivering minoxidil to the blood stream using 5% 5A5. Id. at col.15 line 26. Perhaps because he feared the dangerous cardiac side effects, Rajadhyaksha avoided using this example -it is just a prophetic example. Because Rajadhyaksha lacks the claim limitation, Rajadhyaksha cannot anticipate claims 1, 3, 12, and 14.

Similarly, claim 2 requires "intended for topical use in preventing or treating alopecia," and delivering testosterone blocker "to the depth of the hair bulbs." Rajadhyaksha teaches Example 29, a systemic birth control patch for delivering progesterone to the systemic blood stream. Knowles, *Supplemental Declaration*. It is uncontested that the patch is not used in "alopecia or maintaining healthy hair," nor does it appear to deliver testosterone blocker "to a depth of approximately the depth of hair bulbs." Because it is uncontested that Example 29 lacks these limitations, it cannot anticipate claim 2.

Claims 4 and 8-10 require minoxidil **and** testosterone blocker. It is uncontested that the reference does not disclose this combination. The Examiner, however, appears to misread

these claims to require "either minoxidil **or** progesterone." Office Action at 2, line 20 (27 March 2001).

3. The Reference Fails to Enable the Claimed Invention

For hair loss, the art of record teaches away from combining penetration enhancers with minoxidil or testosterone blocker, due to adverse systemic side effects. This is undisputed. The Examiner, however, notes that the combination - if made- would benefit by using an improved penetration agent. Office Action at 2, lines 18-22 (14 March 2001). While this assertion may be correct, it is inapposite. The reference does not enable the claimed invention. Knowles, *Supplemental Declaration.*

III. SUMMARY

The art of record completely lacks certain claim limitations. It is undisputed that the art of record does not enable the claimed invention. Further, Applicant has "sworn behind" a key reference and the Examiner has refused to produce an affidavit of references. Thus, the pending rejections must be withdrawn.

Please find enclosed (i) a Notice of Appeal; (ii) an Appendix of references relied on; (iii) two additional copies of

this Appeal Brief; (iv) the Supplemental Declaration; and (v) the required fee.

Respectfully submitted,

Mark Pohl, Reg. No. 35,325
25 April 2001

POHL & ASSOCIATE LLP,
PHARMACEUTICAL PATENT ATTORNEYS
55 Madison Avenue, 4th floor (P 4014)
Morristown, NJ 07960 USA
Direct: *licensinglaw@juno.com*
☎ +1 (973) 665-0275

X:\pohlm\Knowles\Appeal.doc

IV. CLAIMS ON APPEAL

1. A composition of matter intended for topical use in preventing or treating alopecia, or maintaining healthy hair, said composition of matter comprising:

   a) an active compound selected from the group consisting of: a pharmaceutically or cosmetically effective topical amount of a testosterone blocker and minoxidil, and

   b) a penetration enhancer, said penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs.

2. The composition of claim 1, wherein said active compound comprises a testosterone blocker.

3. The composition of claim 1, wherein said active compound comprises minoxidil.

4. The composition of claim 3, further comprising a testosterone blocker.

5. The composition of claim 4, wherein the ratio of penetration enhancer to testosterone blocker to minoxidil in the composition is approximately 5 drops : 0.5 grams : 1 gram.

6. The composition of claim 4, wherein said penetration enhancer is trimethyl acetate and wherein said testosterone blocker is progesterone.

7. The composition of claim 5, wherein said testosterone blocker is present in a concentration of 0.5 grams per 4 ounces of finished liquid.

8. The composition of claim 4, labeled for topical cosmetic use in maintaining normal, healthy hair.

9. The composition of claim 4, labeled for topical pharmaceutical use in preventing or treating a disease.

10. The composition of claim 9, wherein said disease comprises alopecia.

11. The composition of claim 4, further comprising a sunscreen in an amount effective to screen radiation.

12. A for preventing or treating alopecia, or maintaining healthy hair, said method comprising:

a) Topically administering an active compound selected from the group consisting of: a pharmaceutically or cosmetically effective topical amount of a testosterone blocker and minoxidil, together with

b) a penetration enhancer, said penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs.

13. The method of claim 12, wherein said active compound comprises a testosterone blocker.

14. The method of claim 12, wherein said active compound comprises minoxidil.

15. The method of claim 14, wherein said active compound further comprises a testosterone blocker.

16. The method of claim 15, wherein the ratio of penetration enhancer to testosterone blocker to minoxidil in the composition is approximately 5 drops : 0.5 grams : 1 gram.

17. The method of claim 15, wherein said penetration enhancer is trimethyl acetate and wherein said testosterone blocker is progesterone.

18. The method of claim 16, wherein said testosterone blocker is present in a concentration of 0.5 grams per 4 ounces of finished liquid.

19. The method of claim 15, labeled for topical cosmetic use in maintaining normal, healthy hair.

20. The method of claim 15, labeled for topical pharmaceutical use in preventing or treating a disease.

21. The method of claim 20, wherein said disease comprises alopecia.

22. The method of claim 15, further comprising a sunscreen in an amount effective to screen radiation.

V. REFERENCES CITED

5

US005994319A

United States Patent [19]

Hoke, Jr.

[11] Patent Number: 5,994,319

[45] Date of Patent: Nov. 30, 1999

[54] **COMBINATION THERAPY FOR ANDROGENIC ALOPECIA WITH ANTISENSE OLIGONUCLEOTIDES AND MINOXIDIL**

[75] Inventor: **Glenn D. Hoke, Jr.**, Mount Airy, Md.

[73] Assignee: **Dyad Pharmaceutical Corporation**

[21] Appl. No.: **08/837,190**

[22] Filed: **Apr. 14, 1997**

Related U.S. Application Data

[60] Provisional application No. 60/015,488, Apr. 15, 1996.

[51] **Int. Cl.**[6] **A61K 48/00**

[52] **U.S. Cl.** **514/44**; 435/6; 514/2; 436/501

[58] **Field of Search** 435/6; 436/501; 514/44; 536/22.1, 23.5, 24.5, 24.3–24.33; 935/77, 78

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,422,262 6/1995 Andersson et al. 435/240.1

OTHER PUBLICATIONS

Drmanac et al. (1990) DNA and Cell Biology, vol. 9, No. 7, pp. 527–534.

Young et al. (1991) Nucleic Acids Res., vol. 19, No. 9, pp. 2463–2470.

Jacobs et al. (1988) Nucleic Acids Res., vol. 16, No. 10, pp. 4637–4650.

Stein et al. (1993) Science, vol. 261, pp. 1004–1012.

New England Biolabs Catalog (1986/87) [Published by New England Biolabs, Beverly, MA, USA], pp. 60–62.

"Finasteride Merck & Co submitted for approval", R&D Focus Drug News, Apr. 7, 1997.

"Merck & Co's Propecia Shows Promise in Hair Loss", Marketletter, Mar. 31, 1997.

Primary Examiner—Ardin H. Marschel

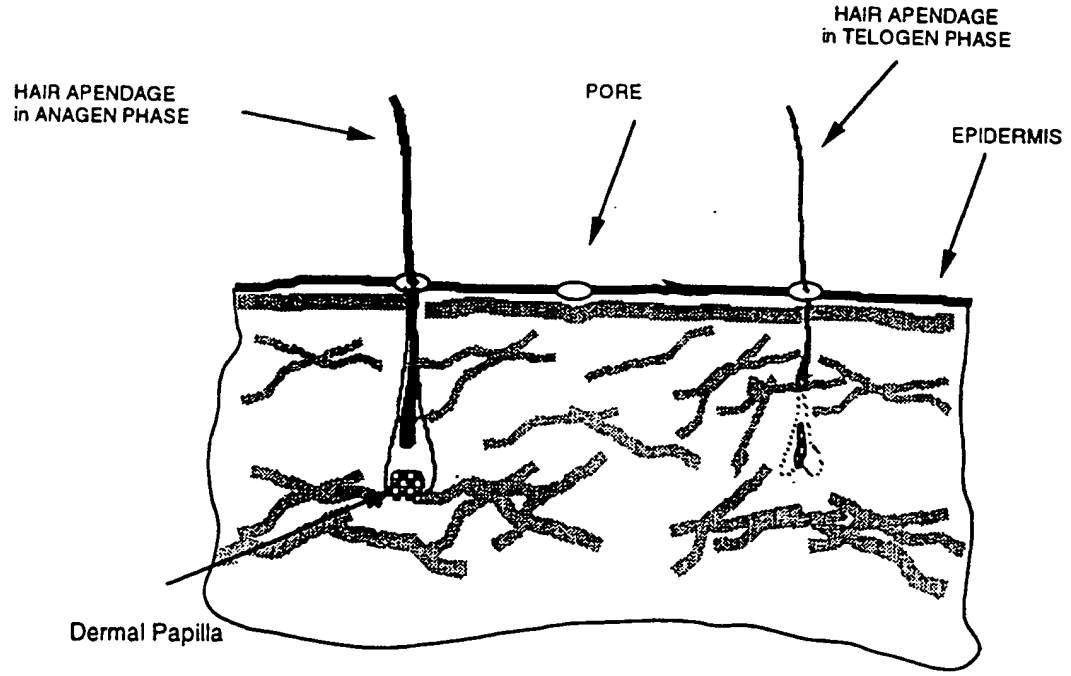
Attorney, Agent, or Firm—Max Stul Oppenheimer

[57] **ABSTRACT**

Minoxidil has been shown to stimulate hair growth or inhibit the loss of hair in a number of patients beginning to develop androgenic alopecia. The mechanism by which minoxidil (2,4-pyrimidinediamine, 6-(1-piperidinyl)-3-oxide) alters the hair growth cycle is uncertain, but is thought to act by increasing vascular circulation to the hair follicle. Inhibitors of steroid metabolism, particularly those that inhibit the conversion of testosterone to dihydrotestosterone, have shown effects on hair cycles, including inhibition of hair loss. One class of enzymes targeted by these inhibitors are the steroid 5-alpha reductases. Minoxidil used in conjunction with effectors of steroid metabolism, leads to enhanced hair growth and decreased rates of hair loss. This specification relates to the use of antisense oligonucleotides targeting 5-alpha reductases used in conjunction with other hair growth enhancers and/or hair loss inhibitors.

6 Claims, 4 Drawing Sheets

Elevated DHT levels cause conversion of anagen hair to telogen hair in andorgenic alopecia



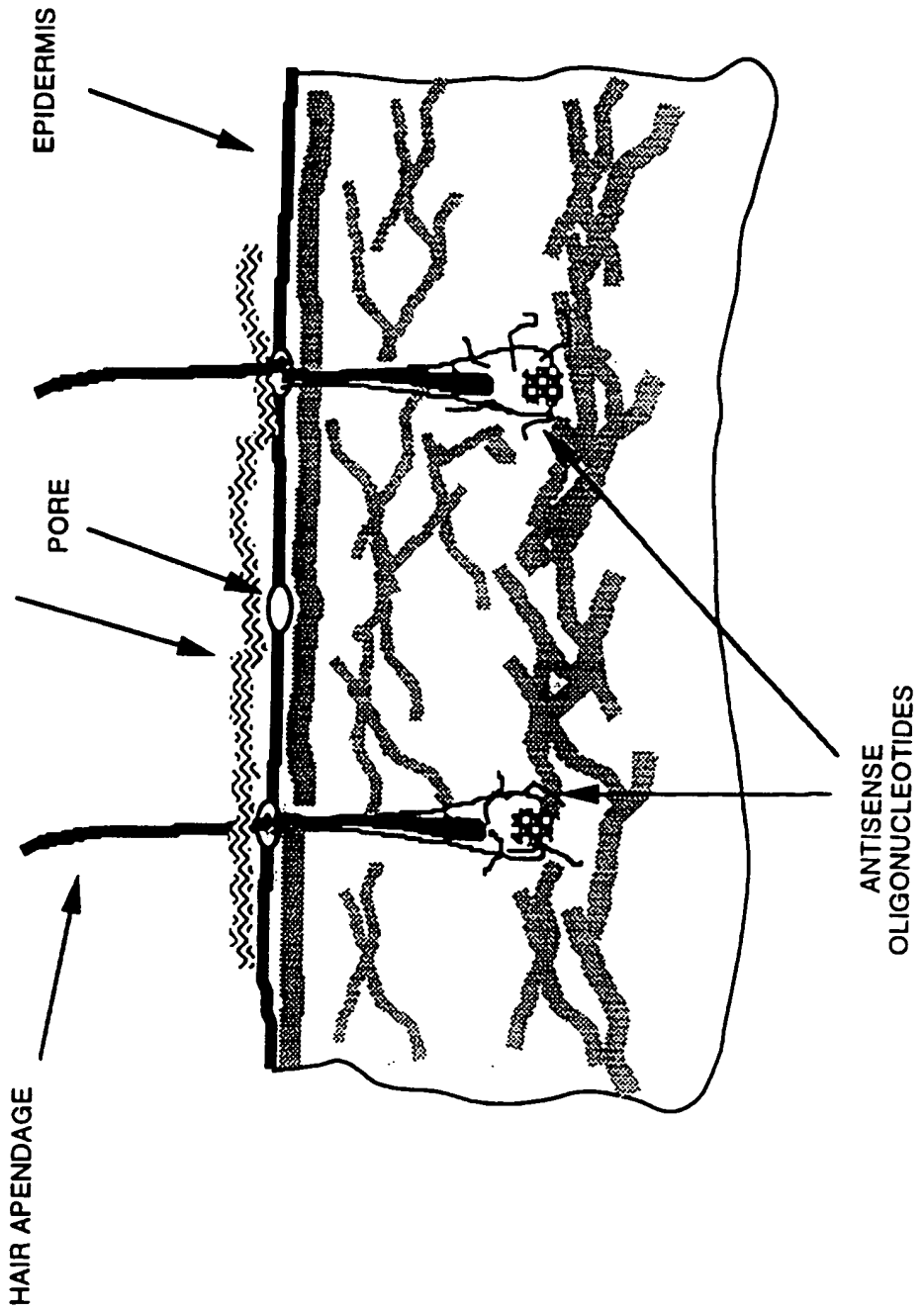
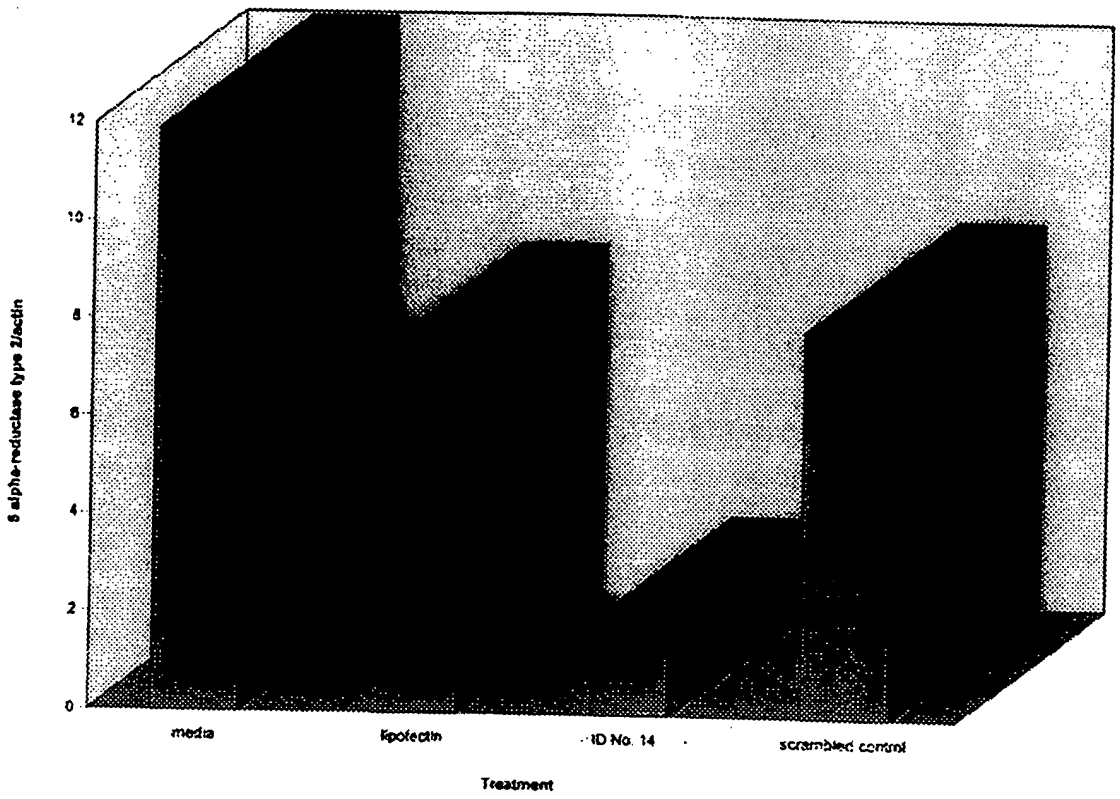


Figure 2. Inhibition of hair loss by antisense targeting 5-alpha reductases and minoxidil

Fig 4 Inhibition of 5 alpha-reductase type 2 by antisense oligonucleotide ID No.14



ing in the reversal of the balding process. (Marketletter Mar. 31, 1997, "Merck & Co's Propecia Shows Promise in Hair Loss").

The Food and Drug Administration has approved the use of a 2% solution of minoxidil as a topical treatment for male pattern baldness. Through a twice daily application, only 8% of patients reported a dense regrowth of scalp hair, while closer to 33% experienced a moderate regrowth. An additional 33% experiencing little or no regrowth or the growth of vellus hair only and the remaining patients experienced no change in their rate of hair regrowth. The mechanism by which minoxidil stimulates hair growth is unknown. Minoxidil is not thought to affect hair growth or loss by acting at the level that androgens exert their effects.

The use of a combination treatment for androgenic alopecia has been suggested for increasing the effectiveness of minoxidil used alone. For example, the combination of minoxidil treatment with finasteride, a 5-alpha reductase inhibitor, demonstrated that, in combination, these two drugs increased the rate of hair regrowth when compared to either drug administered alone (Diani, A. R. et al., 1992, J. Clin. Endocrinol. Metabol. 74: 345–350).

Antisense oligodeoxynucleotides or ribozymes have been successfully employed to decrease mRNA translation (van der Krol, et. al., 1988; Cohen, 1991; Calabretta, 1991; Calabretta, et. al., 1991; Saison-Behmoraras, et. al., 1991). Once the oligonucleotides are taken up by the cells they can elicit an antisense effect by binding to the correct sequences on the target mRNA. The concept behind antisense therapy is based on the assumption that antisense oligonucleotides are taken up by cells and interact with a specific mRNA resulting in the formation of a stable heteroduplex. The interaction of the antisense oligonucleotide with its target mRNA is highly specific and is determined by the sequence of bases complementary to the antisense oligonucleotide as determined by Watson/Crick base pairing.

The development and progression of androgenic alopecia is associated with the local accumulation of DHT. The enzyme steroid 5a-reductase type 1 is expressed in the inner epithelial sheath of the hair follicle (Wolfgang, E. et al., 1994). This enzyme functions to catalyze the conversion of testosterone to dihydrotestosterone. It would appear that the inhibition of steroid 5a-reductase type 1 expression, alone or in combination with other agents that decrease steroid 5a-reductase activity (i.e. Propecia®)) or through the inhibition of the expression of other steroid 5a-reductase genes, would be an effective means for treating androgenic alopecia. It is possible to lower the intracellular concentration of 5a-reductase by reducing the expression of 5a-reductase. Thus, it would be possible to inhibit the conversion rate of testosterone to DHT via 5a-reductase.

Antisense oligonucleotides used for therapeutic purposes were first proposed in 1978 by M. L Stephenson and P. C. Zamecnik (PNAS 75: 280–284). The concept behind antisense therapy relies on the ability of antisense oligonucleotides to be taken up by cells and form a stable heteroduplex with the target mRNA, thereby down regulating the targeted protein's synthesis.

It has been demonstrated in a number of systems by a number of investigators that oligonucleotides containing an antisense sequence targeting a portion of a particular mRNA are capable of hybridizing to the mRNA and inhibiting the translation of the transcript.

The interaction of an antisense oligonucleotide with target mRNA is highly specific, as hybridization is determined by the sequence of bases complementary to the antisense oligonucleotide (Watson/Crick base pairing of the two strands of nucleic acid). This results in multiple points of contact between the antisense oligonucleotide and the mRNA target, which increases the specificity for hybridization to the correct sequence.

Evidence for down regulation of protein synthesis by antisense oligonucleotides has been well documented in vitro (for reviews see van der Krol, A. R., et al. BioTechniques 6: 958–976, 1988; Milligen et. al. J. Med. Chem 36:1923–1937, 1993. In vivo studies using antisense oligonucleotides have demonstrated that injection of radiolabeled antisense oligonucleotides into the blood of mice results in distribution of full-length labeled oligonucleotide to the various tissues. Once in the tissue, oligonucleotides can elicit an antisense effect by binding to the correct mRNA and, thus, be suitable for a therapeutic (Miller, P. S. and Ts'o, P. O. P. Anticancer Drug Design 2: 117–128, 1987).

More specifically, antisense oligonucleotides targeting 5-alpha reductases have demonstrated the capacity to effectively reduce the synthesis of 5-alpha reductase types 1 or 2. These inhibitors are extremely potent, highly selective, and should not exhibit any of the side effects produced by the anti-androgens (i.e., feminization or impotency).

SUMMARY OF THE INVENTION

It is an object of the invention to provide a treatment to reduce hair loss.

It is a further object of the invention to provide a process for restoring hair.

These and other objects are achieved by providing a process for treatment of androgenic alopecia through inhibition of the expression of human steroid 5a-reductase type 1. Specifically, this invention relates to the use of oligonucleotides, or other chemical compositions that interact in a sequence specific manner to either the steroid 5a-reductase type 1 gene, pre-mRNA or mRNA so as to reduce the transcription or translation of the enzyme used in combination with other agents that reduce the rate of hair loss or increase the rate of hair growth. Clinically, such antisense oligonucleotides could be administered alone or in combination with other agents that decrease steroid 5a-reductase activity (i.e. finasteride) or those having other positive effects in the treatment of androgenic alopecia (i.e., minoxidil). The use of such antisense oligonucleotides to control the expression of steroid 5a-reductase type 1, offers the potential of developing highly specific and efficacious therapies for the adjunctive treatment of diseases characterized by the local over production of DHT. Thus, through the use of antisense inhibitors of 5-alpha reductase expression, it would be possible to provide an increased benefit to patients being treated with minoxidil for androgenic alopecia.

In a preferred embodiment of the invention, therapeutically effective amounts of oligonucleotides can be administered to a patient so as to substantially block the tissue-specific transcription of the human steroid 5a-reductase type 1 (or type 2) gene or the translation of the steroid 5a-reductase type 1 (or type 2) mRNA transcript, thereby substantially reducing the levels of dihydrotestosterone (DHT) in the patient who expresses a condition characterized by the local over production of DHT. It has been demonstrated recently that the use of finasteride (a 5-alpha reductase which blocks the conversion of testosterone to dihydrotestosterone) miniaturizes scalp hair follicles, resulting in the reversal of the balding process. (Marketletter Mar. 31, 1997, "Merck & Co's Propecia Shows Promise in Hair

the 3'-untranslated region of the steroid 5a-reductase type 1 or 2 mRNA transcripts. In addition to the sequences described above, other sequences contained within the 5a-reductase transcripts are targeted. This strategy has been adopted because, as yet, there is no method currently available that can predict, with precision, sequences that will become effective therapeutics. Moreover, this invention further contemplates antisense oligonucleotides made complementary to any portion of the steroid 5a-reductase genes and which are capable of cross-linking DNA, intercalating DNA or binding more tightly by mechanisms such as, for example, triple stranding. Furthermore, the invention contemplates that any oligonucleotide capable of substantially inhibiting the expression of steroid 5a-reductase type 1 or 2 can be used.

Oligonucleotides of varying lengths have been successfully used to inhibit gene expression. For example, in U.S. Pat. No. 4,806,463 oligonucleotides ranging in size from 12 bases to 26 bases were shown to be incorporated by cells and to be capable of inhibiting the expression of a target mRNA.

In order for the described antisense oligonucleotides to function therapeutically, the oligonucleotides or modified oligonucleotides must be taken up by the cell that expresses the target gene, pre-mRNA, or mRNA. The oligonucleotides of the present invention are constructed so as to insure that the oligonucleotide will pass through the plasma membrane and achieve an intracellular concentration that is sufficient to decrease the expression of steroid 5a-reductases. Oligonucleotides that are constructed to bind to the steroid 5a-reductase type 1 or 2 genes are further modified, if necessary, to enable them to pass through the nuclear membrane in levels that are sufficient to reduce transcription. Recent attempts at enhancing the cellular uptake of antisense oligonucleotides have employed a wide variety of techniques including the use of lipoproteins, (de Schmidt, et. al., 1991), and a wide variety of conjugates, such as poly-L-lysine and cholesterol (Goodchild, 1990). Conjugation of cholesterol to the 5' end of an oligonucleotide has been reported to result in a molecule that exhibited reduced serum clearance due to reduction in renal excretion, compared to that observed with control oligo deoxynucleotides (ODNs) (de Schmidt, et. al., 1991). As a result, the conjugation of cholesterol to ODNs may allow an increase in the delivery of drug to liver cells via the LDL transport mechanism. Liposomes containing antisense oligonucleotides can also be targeted to specific cell types by the addition of cell-specific antibodies (Leonetti, et. al., 1990). These and other methods of achieving and maintaining adequate intracellular concentrations of the oligonucleotides are contemplated by this invention and include other methods and compositions that have the capacity to enhance cellular uptake or decrease the efflux of internalized oligonucleotides. Such modifications should not alter the specificity of the oligonucleotide for its target sequence.

The oligonucleotides of this invention comprise predetermined sequences of DNA ranging in size from about 3 bases up to about 100 bases, which is sufficient to define a unique sequence in one of the human steroid 5a-reductase target transcripts. Less than 10 bases may be used, however the degree of sequence specificity for the mRNA transcripts that encode human steroid 5a-reductases decreases rapidly with decreasing lengths of the oligonucleotides. On the other hand, oligonucleotide sequences greater than about 100 bases may be subject to decreased uptake by cells. It is preferable that the oligonucleotides comprise about 12 to 26 bases. In a most preferred embodiment a 15 to 25-mer oligonucleotide is used.

Antisense oligonucleotides that are intended for use as drugs must achieve sufficient concentrations in order to decrease the expression of a target protein in a manner that provides therapeutic benefit. The oligonucleotides contemplated in this invention are constructed, or otherwise modified, so as to increase their stability by enhancing resistance to various degradative enzymes (e.g., nucleases). Such modifications will function to permit the concentration of the oligonucleotide therapeutic to be maintained at a level that is sufficient so as to realize therapeutic benefit but cannot substantially alter the specificity of the oligonucleotide for its target sequence. Modifications that improve oligonucleotide stability or efficacy include but are not limited to modifications to the phosphate backbone, termini, sugar moieties and the individual nucleic acid bases. Conjugations to peptides, proteins, carbohydrates, lipids, vitamins or any other conjugation that increases therapeutic potency or efficacy can also be used. Also, any modifications resulting in stable secondary structures including circularization of the oligonucleotide and target sequence, and intrastrand joining of the 3' to the 5' termini through covalent bonds or hybridization and triple stranded binding to mRNA can also be made. Any modifications that reduce nuclease sensitivity while substantially maintaining the affinity and substrate specifically and solubility exhibited by unmodified oligonucleotides are within the scope of the invention.

Several chemically modified oligonucleotides have been developed which substantially block or improve resistance to nuclease activity. These oligonucleotide modifications include phosphorothioate oligonucleotides wherein one of the phosphate oxygens is replaced by sulfur. Another type of modification of oligonucleotides is accomplished by replacing the charged phosphate oxygen with a methyl group or other alkyl group. These nonionic DNA analogs include, for example, methyl phosphonates, alkyl-phosphorothioates, and O-alkyl phosphotriesters. A preferred O-alkylphosphotriester is O-methylphosphotriester. Other DNA backbone modifications at the phosphate group include for example, phosphorodithioate, and phosphotriester oligonucleotides or oligonucleotides based on protein-nucleic acid structures or morpholino-like structures.

Various chemical modifications to either or both the 3'- or 5'-termini and the individual nucleic acid bases are known to improve stability of oligonucleotides to nucleases, stabilize the interaction of oligonucleotides with their specific target molecule, or enhance uptake of the oligonucleotides by cells. Moreover, chemical modifications to the 3' or 5' termini or modifications internal to the oligonucleotide can also be introduced as reporter molecules for example, to allow tracking of the oligonucleotide or as lipophilic moieties to enhance cell uptake. Such molecules can be introduced to both unmodified and backbone modified synthetic oligonucleotides. These moieties can be introduced for example, through thio or amino linkages to terminal hydroxyl or phosphate groups or to specific bases.

Other modifications to the oligonucleotides contemplated in this invention include for example, DNA intercalators, photochemically activated cross-linking or cleaving agents, alkylating agents and redox active nucleic acid cleaving groups.

In vivo and in vitro studies of the degradation of chemically modified oligonucleotides have clearly illustrated that modifications to the phosphate backbone, termini, sugar moiety and individual nucleic acids improve oligonucleotide efficacy or stability or both (Goodchild, 1990). Moreover, acute toxicity studies in mice have demonstrated that some modified oligomers are tolerated at about the same concentrations without undesirable side effects as unmodified oligomers.

of 0.001 to 10.0 mM. In the transient transfection assay, the oligonucleotides are either co-transfected with the steroid 5a-reductase cDNA or added to the medium at a concentration of 0.001 to 10.0 mM following transfection. Cells are plated in multi-well tissue culture plates. The size of the well used for a particular assay is determined by the level of steroid 5a-reductase expressed by a given cell line.

The substrate is prepared by dissolving unlabeled testosterone (Sigma Chemical Co., St. Louis, Mo.) in absolute ethanol followed by the addition of either [7-^{3}H (N)]-testosterone (23.3 Ci/mmole) or [^{14}C]-testosterone (50 mCi/mmol) (New England Nuclear, Boston, Mass.). The solvent is evaporated under a stream of nitrogen and the steroids reconstituted in an appropriate medium.

The medium in the sample wells is aspirated and replaced with fresh medium containing the radiolabeled substrate. An additional three wells containing medium and substrate but no cells is also included in order to account for the non-enzymatic metabolism of the substrate. The plates are returned to the incubator and incubated for an appropriate incubation period that is again dependent on the level of steroid 5a-reductase expressed by the cell line.

At the end of the incubation period the medium is collected and transferred to an extraction tube containing 5 ml of toluene-ethanol (9:1), to which has been added 40–250 mg each of unlabeled carrier steroids (estriol, estradiol, estrone, 5a-androstan-3a,17b-diol, 5a-androstan-3b,17b-diol, 4-androstene-3,17-dione, 5a-androstan-3,17-dione, testosterone, and 5a-dihydrotestosterone (Steraloids, Inc. Wilton, N.H.). Depending upon the method used to detect the radiolabeled steroids the extraction solvent may or may not contain 1,000 and 10,000 dpm of [4-^{14}C]-dihydrotestosterone (steroid 50–60 mCi/mmol) and [4-^{14}C]-testosterone (50 mCi/mmol) (New England Nuclear, Boston, Mass.); respectively. In assays that employ [7-^{3}H (N)]-testosterone as a substrate, the [^{14}C]-steroids are included as recovery standards to quantify procedural losses. A small amount of NaCl is also added to the extraction tubes to prevent foaming. The samples are vortexed for approximately 30 seconds and then centrifuged for 10 minutes at 500× g. The organic phase is collected and the solvent evaporated. The steroids are then reconstituted in dichloromethane-methanol (9:1) and analyzed by thin layer chromatography

The extracted samples are applied to silica gel 60F_{254}, 0.25 mm thick, thin layer chromatography plates (EM Science, Cincinnati, Ohio). The plates are developed in a solvent system consisting of chloroform-ethyl acetate (3:1, Mallinckrodt Inc. Paris, Ky.). The plates are allowed to developed until the solvent front migrates to within 2.0 cm of the top of the plate. After removal from the tanks the plates are air dried. The plates are then viewed under 254 nm UV light and the visible spots marked. The plates are then sprayed with primulin (0.001% in acetone-water (4:1) according to the method of Wright (Moore and Wilson, 1975) which allows the identification of additional steroids under 365 nm UV light. The spots are scraped from the plate using a glass wool plugged Pasteur pipette attached to a vacuum line. The steroids are eluted directly into scintillation vials by the addition of 0.2 ml of dichloromethane followed by two washes of 2.0 ml of methanol. The organic solvent is evaporated, and 10.0 ml of scintillation fluid (Ready Organic, Beckman Instruments, Inc. Fullerton Calif.) are added. Samples are analyzed by liquid scintillation spectrometry. In assays that employ [^{14}C]-testosterone as the substrate, steroid metabolism is analyzed directly using the PhosphorImager imaging system (Molecular Dynamics, Inc., San Jose, Calif.).

Following removal of the media for extraction, the cells are washed with phosphate buffered saline (PBS, pH 7.4), and then harvested by exposure to a trypsin-EDTA solution (0.025% trypsin, 0.265 mM EDTA). The cells are collected and centrifuged at 1400× g for 5 minutes. The supernatant is decanted and the cells resuspended in PBS. An aliquot of the cell suspension is counted in a Coulter Counter Model ZM (Coulter Electronics, Ltd., Luton Beds, England). The remaining cells are sonicated and the protein determined according to the method of Bradford (Bradford, 1976). Corrections are made for procedural losses, and the data expressed as percent inhibition based on steroid concentration in terms of picomoles per mg protein or picomoles/10^5 cells.

Example 3

It is possible to analyze the proteins of treated and control cells using SDS- denaturing polyacrylamide gel electrophoresis followed by the transfer of resolved proteins to a solid support (Western transfer), such as nitrocellulose, or other filter supper. Filter blots are blocked with non-specific proteins and then analyzed using a primary antibody that recognizes either 5-a reductase type 1 or 2. Following the primary antibody, the blots are then reacted with a second antibody that binds to the first antibody and has incorporated with in the second antibody a reporter moiety (i.e., radioactivity, enzyme linked, or other easily detectable reporter group). Following treatment with the second antibody, the blot is analyzed for the amount of 5-a reductase present in a given amount of cellular protein. Thus, it is possible to actually quantitate the amount of 5-a reductase being expressed in cells.

Chinese hamster ovary cells transfected with the human 5aR-I gene (CHO 1827) or the human 5-aR-II gene (CHO 1829) are plated at 8×106 cells per well, in 6 well dishes in DMEM/FI2(1:1) and 5% Fetal Bovine Serum (FBS). Cells are dosed with ODN's (containing 5 ug/ml LipofectinTM, Life Technologies, Inc. Gaithersburg, Md.) in serum-free Opti-MEM for 3 hr at 37° C. and then returned to serum-containing media. Dosing is carried out for three consecutive days due to the long half-life of the 5aR proteins. Cells are harvested by detachment in PBS with 5 mM EDTA, pelleted, and lysed in a NP40-RIPA buffer. Cellular proteins (5 ug) are then separated by SDS-PAGE on a 12% acrylamide gel before transferring onto a PVDF membrane. The amount of the 5-aR-I or the 5aR-II proteins and actin are then detected by direct visualization using ECL-Western (Amersham, Arlington Heights, Ill.) and antibodies specific for the proteins. Actin is included as an internal control and all results are presented as the ratio of 5aR signal relative to the actin signal. Quantitation is performed using a scanning densitometer and values are compared to controls. FIG. 3 shows the inhibition of 5-aR type I using antisense oligonucleotide Seq. ID No. 5 relative to controls that are untreated, those receiving lipofectin, or a scrambled control oligonucleotide. FIG. 4 shows the inhibition of 5-aR type II using antisense oligonucleotide Seq. ID No. 14 relative to controls that are untreated, those receiving lipofectin, or a scrambled control oligonucleotide.

While a specific embodiment of the invention has been shown and described in detail to illustrate the application of the principles of the invention, it will be understood that the invention may be embodied otherwise without departing from such principles and that various modifications, alternate constructions, and equivalents will occur to those skilled in the art given the benefit of this disclosure. Thus, the invention is not limited to the specific embodiment

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCGCCGTTGC CATCGCCAGG G 21

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCCCGTCGCC GTTGCCATCG C 21

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGCGCTCCTC CGCCACCCCC G 21

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GACAGACCAG CTGGCCAGGG C 21

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCGCCATTGG AAAGCTTCAA G 21

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GCGTATTTAG GTACTTATTA G 21

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTGCATCGCG CCGTGTTCCT C 21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGCACTGAAC CTGCATCGCG C 21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AGGATCCCCG CGGGCACCGC G 21

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGGGTCTTTG TGGCTTCAGA G 21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GCCACATGTA CTTGGATTGC C 21

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

[11] Patent Number: 5,053,403
[45] Date of Patent: Oct. 1, 1991

Orentreich et al.

[54] METHODS FOR TREATMENT OF MALE-PATTERN BALDNESS

[75] Inventors: Norman Orentreich, 140 E 72, New York; Jonathan R. Matias, Richmond Hill, N.Y.

[73] Assignee: Norman Orentreich, New York, N.Y.

[21] Appl. No.: 438,979

[22] Filed: Nov. 20, 1989

Related U.S. Application Data

[60] Continuation of Ser. No. 79,609, Jul. 30, 1987, abandoned, which is a division of Ser. No. 846,498, Mar. 27, 1986, Pat. No. 4,684,635, which is a continuation of Ser. No. 609,152, May 11, 1984, abandoned.

[51] Int. Cl.[5] A61K 31/56

[52] U.S. Cl. 514/170; 514/171; 514/175; 514/177; 514/178; 514/181

[58] Field of Search 514/170, 171, 175, 177, 514/178, 181

[56] References Cited

U.S. PATENT DOCUMENTS

4,684,635 8/1987 Orentreich et al. 514/170

OTHER PUBLICATIONS

Aron-Brunetiere-"Aspects of Endocrinological Treatment", Hair Research (1981)pp. 312-317.

Matias, J. R., Malloy, V. & Orentreich, N., "Animal Models of Androgen-Dependent Disorders of the Pilosebaceous Apparatus," *Arch. Dermatol. Res.*, 281: 247-53 (1989).

Nielsen,. P. G., "Treatment of Moderate Idiopathic Hirsutism with a Cream Containing Canrenone" (an Antiandrogen), *Dermatologica*, 165, pp. 636-639 (1982).

Rentoul, J. R., "Management of the Hirsute Woman," *Intl. J. Derm.*, 22, pp. 265-72 (1983).

Primary Examiner—Leonard Schenkman
Attorney, Agent, or Firm—Panitch Schwarze Jacobs & Nadel

[57] ABSTRACT

Synergistic compositions for inhibiting the action of androgens comprise the combination in a single topical preparation of a 5α-reductase enzyme inhibitor and an androgen receptor blocking agent in a pharmaceutically and dermatologically acceptable vehicle. The compositions may be topically applied to affected areas of the skin in the treatment or prevention of sebaceous gland hypertrophy, hirsutism and male-pattern baldness in mammals. The inhibitor and blocking agent are present in the composition in a weight ratio of about 1:20 to 5:1, with the inhibitor comprising up to about 0.1% w/v of the comopsition and the blocking agent comprising up to about 1% w/v of the composition.

9 Claims, No Drawings

METHODS FOR TREATMENT OF MALE-PATTERN BALDNESS

This is a continuation of Ser. No. 07/079,609, 07/30/87 abandoned which is a division, of application Ser. No. 846,498, now U.S. Pat. No. 4,684,635, filed Mar. 27, 1986, which is a continuation of application Ser. No. 609,152, filed May 11, 1984 abandoned.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for inhibiting the action of androgens. More particularly, the invention is directed to synergistic combinations of a 5α-reductase enzyme inhibitor and an androgen receptor blocking agent for topical applications to the skin in the treatment and prevention of sebaceous gland hypertrophy, hirsutism and male-pattern baldness.

BACKGROUND OF THE INVENTION

The development of acne, hirsutism and male-pattern baldness is dependent upon the presence of androgens, particularly testosterone and dihydrotestosterone (DHT). Testosterone is secreted into the blood stream by the adrenals and gonads and enters the cells of the sebaceous glands or hair follicles. This steroid binds specifically to the 5α-reductase enzyme which converts testosterone to its most active metabolite DHT. DHT binds to specific receptor proteins in the cell cytoplasm, and this steroid-protein complex is translocated to the nucleus of the cell where DHT becomes bound to the nuclear receptor protein. Nuclear binding is followed by the synthesis of specific classes of proteins, eventually leading to hypertrichosis (hirsutism), alopecia (male-pattern baldness) or sebaceous gland hypertrophy (manifested as acne or other skin inflammations).

The inhibition of testosterone conversion to DHT by the 5α-reductase enzyme and the inhibition of DHT binding to the receptor protein are accepted therapeutic modalities. A number of compounds, called antiandrogens, have been developed which can interfere with either testosterone metabolism or DHT binding to the receptor.

The serious side effects (such as decreased libido) produced by the systemic administration of antiandrogens preclude the systemic use of these drugs for the treatment of the above skin disorders. For example, progesterone is a highly active 5α-reductase enzyme inhibitor, but systemically disturbs the menstrual cycle in women, since it must be used on a regular basis in order to be effective. Many studies have shown that individual antiandrogens can be used topically to inhibit the action of androgens. However, applicants are not aware of any prior studies on the effectiveness of combining 5α-reductase inhibitors with androgen receptor blocking agents in a topical preparation.

BRIEF SUMMARY OF THE INVENTION

It has been discovered that certain combinations of a 5α-reductase enzyme inhibitor and an androgen receptor blocking agent have a synergistic effect in the inhibition of the action of androgens particularly in the treatment and prevention of sebaceous gland hypertrophy, hirsutism and male-pattern baldness. The compositions of the invention are applied topically to affected areas of the skin of humans and other mammals in therapeutically effective amounts. These combinations of inhibitors and blocking agents at certain concentrations and ratios act synergistically to produce a marked inhibition of the effects of the endogenous androgens on the skin. The steroid combinations may be delivered through the skin by means of various topical vehicles.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

There are a number of steroids, steroid precursors and derivatives which are known to be effective as 5α-reductase inhibitors and which may be used in the compositions of the present invention. These include progesterone, 17β-carboxylic acid derivatives of testosterone, desoxycorticosterone, desoxycorticosterone acetate, 19-nor-testosterone, 4-pregnen-20β-ol-3-one and 17α-hydroxyprogesterone. Of these, progesterone is preferred since it is the most active inhibitor and has been shown to be topically effective by itself.

Both steroidal and non-steroidal androgen receptor blocking agents (blockers or inhibitors) may be used in the synergistic compositions of the present invention. Representative examples of steroidal blocking agents include spironolactone, cyproterone acetate, trimethyltrienolone (available from Roussel Uclaf under the designation RU 2956), canrenone and canrenoic acid. Examples of suitable non-steroidal blocking agents include flutamide (α,α,α-trifluoro-2-methyl-4'-nitro-m-propionotoludide) and hydroxy-flutamide (α,α,α-trifluoro-2-methyl-4'-nitro-m-lactoluidide), both available from Schering Corp., and RU 23908 (5,5-dimethyl-3-[4-nitro-3(trifluoromethyl)phenyl]-2,4-imidazolidinedione) and RU 22930 (5,6-dihydro-2-methyl-4-[4-nitro-3-(trifluoromethyl)phenyl]-2H-1,2,4-oxadiozin-3-(4H)-one, available from Roussel Uclaf.

Generally, the ratios of 5α-reductase inhibitor to androgen receptor blocking agent which have been found to be effective in the compositions of the present invention range between about 1:20 and 5:1. Ratios of about 1:1 to 2:1 are preferred.

It has also been found that the synergistic effects of the combinations of the present invention are most pronounced at relatively low concentrations of the 5α-reductase inhibitor and androgen receptor blocker. In particular, optimal concentrations for the 5α-reductase inhibitors appear to range from about 0.005% to 0.1% weight/volume of the total composition, while optimal concentrations for the androgen receptor blockers appear to range from about 0.01% to 0.5% weight/volume of the total composition. Concentrations below about 0.005 5α-reductase inhibitor and about 0.01 androgen receptor blocker give very little antiandrogenic response. Concentrations above about 0.1% 5α-reductase inhibitor and about 0.5% androgen receptor blocker are not only unnecessarily wasteful, expensive and liable to produce adverse side effects, but were also found to have very little synergistic effect above that obtained by applying either the 5α-reductase inhibitor or the androgen receptor blocker alone.

It has been found according to the present invention that when a 5α-reductase inhibitor is applied together with an androgen receptor blocker as a single topical preparation, the resulting inhibition of androgens was greater than the sum total of the effects produced independently by the 5α-reductase inhibitor and the androgen receptor blocker.

These effects will now be demonstrated and described in more detail with reference to the following specific, non-limiting examples. The hamster ear seba-

ceous gland was selected as a test model because of the similarities in morphology to human sebaceous glands and in all turnover time. It is well accepted in the medical literature that antiandrogens which work to inhibit sebaceous glands by topical means should also work to inhibit the androgenic effects in the hair follicle. Thus, the mechanism of androgenic control is similar for sebaceous gland hypertrophy, hirsutism and androgenic alopecia.

EXAMPLE NOS. 1-12

A series of experiments were carried out using 5 or 6 animals for each data point. The various concentrations of 5α-reductase inhibitor or androgen receptor blocker or both as shown in Table I where each dissolved in acetone and 25 μl of each solution was applied unilaterally on the right ventral ear skin of adult male Syrian hamsters two times a day, five days per week for a total duration of four weeks. Control animals received topical applications of acetone alone. At the end of the experiment, the androgen-sensitive ear skin sebaceous glands of the hamsters were analyzed according to the method of Matias and Orentriech, *Journal of Investigative Dermatology*, 81:43 (1983), with minor modifications. In brief, the ventral ear skin was manually separated from the cartilage and stained for three hours with 0.1% Sudan Black in propylene glycol. After rinsing overnight with 85% propylene glycol, a defined area (medial zone; 5-8 mm from the apex of the ear) was biopsied. The darkly stained sebaceous glands were visualized from the underside at a magnification of 625X and quantitated planimetrically using a graphics computer interfaced with the microscope.

The size of the sebaceous glands taken as above from the right ventral ear of each hamster was compared with similar samples taken from the acetone treated right ventral ear of another group of hamsters as controls. The effects of the various inhibitors, as measured by sebaceous glands size, are given in Table I as a percent of the size of the vehicle treated control group. That is, 100% means that the treated ear sebaceous glands were the same size as the sebaceous glands of the vehicle treated control ears, and hence there was no inhibition of androgenic activity. On the other hand, an androgenic activity of 60% means that the sebaceous gland size of the treated ear was 60% of the size of the sebaceous glands of the vehicle treated control ear, thus indicating a 40% decrease in androgenic activity. Generally, a percent activity between about 90% and 100% is considered an insignificant inhibition of androgenic effects on sebaceous glands.

The data in Table I demonstrate that each combination of 5α-reductase inhibitor and androgen receptor blocker produces a synergistic effect as compared to the effects of 5α-reductase inhibitor and androgen receptor blocker alone. That is, the sum of the individual inhibitory activities (100 minus androgenic activity) of the 5α-reductase inhibitor and androgen receptor blocker is considerably less than the inhibitory activity of the combined composition of 5α-reductase inhibitor and androgen receptor blocker. Such reductions in androgen activity correlate very well to inhibition of acne, hirsutism and male-pattern baldness.

TABLE I

THE SYNERGISTIC EFFECTS OF VARIOUS COMBINATIONS OF 5α-REDUCTASE AND ANDROGEN RECEPTOR INHIBITORS

EFFECT OF 5α-REDUCTASE INHIBITOR ALONE			COMBINED	EFFECT OF ANDROGEN RECEPTOR BLOCKER ALONE		
5α-REDUCTASE INHIBITOR	CONCENTRATION (% w/v)	% OF CONTROL GLAND SIZE	% OF CONTROL GLAND SIZE	% OF CONTROL GLAND SIZE	CONCENTRATION (% w/v)	ANDROGEN RECEPTOR INHIBITOR
PROGESTERONE	0.05	92	60	85	0.5	SPIRONOLACTONE
PROGESTERONE	0.10	77	57	92	0.1	SPIRONOLACTONE
PROGESTERONE	0.05	92	65	92	0.1	SPIRONOLACTONE
PROGESTERONE	0.010	100	65	92	0.1	SPIRONOLACTONE
PROGESTERONE	0.005	100	73	92	0.1	SPIRONOLACTONE
PROGESTERONE	0.05	92	75	86	0.05	SPIRONOLACTONE
PROGESTERONE	0.025	100	52	86	0.05	SPIRONOLACTONE
PROGESTERONE	0.010	100	74	92	0.05	SPIRONOLACTONE
PROGESTERONE	0.005	100	85	100	0.010	SPIRONOLACTONE
PROGESTERONE	0.05	92	68	81	0.05	FLUTAMIDE
PROGESTERONE	0.05	92	54	93	0.05	TRIMETHYLTRIENOLONE
PROGESTERONE	0.05	92	66	94	0.05	CYPROTERONE ACETATE

The compositions of the present invention may be applied in any of a wide variety of topical application forms, including solutions such as the acetone solution used in the examples above, tinctures, creams, ointments, gels, lotions or aerosol sprays. Such preparations may be either alcohol- or water-based or a combination of alcohol/water base. Typical examples of topical preparations according to the present invention are set forth in Examples 13-18 below.

EXAMPLE 13

Solution

	% w/v
Progesterone	0.025
Spironolactone	0.05
Acetone	QS
	100.0

EXAMPLE 14

Tincture

	% w/v
Progesterone	0.025
Canrenone	0.05
Propylene glycol	10.0
Wager	24.0
Alcohol	QS
	100.0

EXAMPLE 15

Cream

	% w/v
Desoxycorticosterone	0.025
Trimethyltrienolone	0.05

Oil Phase

Petrolatum	10.0
Stearyl alcohol	4.0
Polyethylene glycol monostearate	4.0
Stearic acid	2.0

Water Phase

Glycerin	5.0
Triethanolamine	1.0
Preservative (methyl and Propyl parabens)	0.2
Water	QS
	100.0

EXAMPLE 16

Ointment

	% w/v
Progesterone	0.025
Flutamide	0.05
Propylene glycol	12.0
Sorbitan sesquioleate	4.0
Petrolatum	QS
	100.0

EXAMPLE 17

Gel

	% w/v
Desoxycorticosterone acetate	0.025
Non-steroidal antiandrogen RU 23908)	0.05
Carbomer 940	1.0
Triethanolamine	0.4
Isopropyl myristate	5.0
Water	35.0
Alcohol	QS
	100.0

EXAMPLE 18

Aerosol Lotion

	% w/v
Progesterone	0.025
Canrenoic acid	0.05
Polyethylene glycol monostearate	2.0
Myristyl myristate	2.0
Polysorbate 20	1.0
Water	QS
Alcohol	12.0
Dimethyl ether propellent	10.0
Fluorohydrocarbon propellent	10.0
	100.0

It will be recognized by those skilled in the art that changes may be made to the above-described embodiments of the invention without departing from the broad inventive concepts thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover all modifications which are within the scope and spirit of the invention as defined by the appended claims.

We claim:

1. A method for treating male-pattern baldness in mammals comprising topically applying to the affected areas of the skin therapeutically and synergistically effective amounts of a composition comprising (a) an inhibitor of the conversion of testosterone to dihydrotestosterone by the 5α-reductase enzyme, (b) a blocking agent which blocks the binding of dihydrotestosterone to receptor protein in cell cytoplasm, and (c) a pharmaceutically and dermatologically acceptable vehicle for said inhibitor and said blocking agent.

2. A method according to claim 1 wherein said inhibitor and said blocking agent are present in the weight ratio of about 1:20 to 5:1.

3. A method according to claim 1 wherein said inhibitor and said blocking agent are present in the weight ratio of about 1:1 to 1:2.

4. A method according to claim 1 wherein said inhibitor comprises up to about 0.1% and said blocking agent comprises up to about 1.0% of the composition, said percentages being on the basis of weight/volume.

5. A method according to claim 1 wherein said inhibitor is selected from the group consisting of progesterone, 17β-carboxylic acid derivatives of testosterone, desoxycorticosterone, desoxycorticosterone acetate, 19-nortestosterone, 4-pregnen-20β-ol-3-one and 17α-hydroxyprogesterone.

6. A method according to claim 1 wherein said inhibitor is progesterone.

7. A method according to claim 1 wherein said blocking agent is selected from the group consisting of spironolactone, cyproterone acetate, flutamide, trimethyltrienolone, hydroxy-flutamide, canrenone, canrenoic acid, 5,5-dimethyl-3-[4-nitro-3(trifluoromethyl)-phenyl]-2,4-imidazolidinedione and 5,6-dihydro-2-methyl-4-[4-nitro-3-(trifluoromethyl)phenyl]-2H-1,2,4-oxadiozin-3-(4H)-one.

8. A method according to claim 1 wherein said vehicle comprises water, alcohol, acetone, or a combination thereof.

9. A method according to claim 1 wherein said vehicle is selected from the group consisting of solutions, tinctures, creams, ointments gels, lotions and aerosol sprays.

* * * * *

United States Patent [19]

Bazzano

[11] Patent Number: **5,183,817**

[45] Date of Patent: **Feb. 2, 1993**

[54] **COMBINATIONS OF RETINOIDS AND MINOXIDIL-TYPE COMPOUNDS FOR HAIR GROWTH**

[76] Inventor: **Gail S. Bazzano**, 4506 Avron Blvd., Metairie, La. 70006

[21] Appl. No.: **283,646**

[22] Filed: **Dec. 13, 1988**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 136,525, Dec. 22, 1987, abandoned, which is a continuation of Ser. No. 463,146, Feb. 2, 1983, abandoned, which is a continuation-in-part of Ser. No. 235,169, Feb. 17, 1981, abandoned, and a continuation-in-part of Ser. No. 318,607, Nov. 9, 1981, abandoned, and a continuation-in-part of Ser. No. 368,730, Jun. 9, 1982, abandoned, and a continuation-in-part of Ser. No. 414,854, Sep. 3, 1982, abandoned.

[51] Int. Cl.[5] **A61K 31/505**; A61K 31/07

[52] U.S. Cl. **514/256**; 514/725; 514/880

[58] Field of Search 514/725, 256, 313

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,382,248	5/1968	Anthony et al.	514/880
3,461,461	8/1969	Anthony et al.	514/880
3,464,987	9/1969	Ursprung et al.	514/880
3,729,568	4/1973	Kligman	514/859
3,882,244	5/1975	Lee	424/70 UX
3,973,016	8/1976	Morrison	514/79
4,139,619	12/1979	Chidsey	514/725 UX
4,170,229	10/1979	Olson	424/70 UX
4,220,772	9/1980	Muller et al.	544/255
4,232,438	3/1982	Peck	424/70 UX
4,247,547	1/1981	Marks	424/70 UX
4,287,338	9/1981	McCall	544/123
4,304,787	12/1981	Gander et al.	424/70 UX
4,333,924	6/1982	Bowley et al.	424/70 UX

FOREIGN PATENT DOCUMENTS

158799	9/1954	Australia	424/70 UX
553262	12/1956	Belgium	424/70 UX
0242967	3/1987	Fed. Rep. of Germany	514/859
51-73137	6/1976	Japan .	
55-9007	1/1980	Japan	514/313
906000	11/1960	United Kingdom	514/859
1466062	2/1977	United Kingdom	514/313

OTHER PUBLICATIONS

Merck Index 9th edition, 1979, pp. 1060, 1287 to 1289.

Primary Examiner—Dale R. Ore
Attorney, Agent, or Firm—Panitch Schwarze Jacobs & Nadel

[57] **ABSTRACT**

Increase in the rate of hair growth, stimulation of hair follicles to produce new hair growth, prolongation of the anagen phase of the hair cycle, conversion of vellus hair to growth as terminal hair, and treatment of alopecias due to organic dysfunction of the hair follicle is attained in mammalian skins by either oral administration or by topical application to the skin, hair and/or hair follicles of the mammal of effective amounts of a retinoid, particularly retinoic acid, and a minoxidil-type compound. The combination may be administered or applied alone or with other adjunctive compounds including vitamins, such as Vitamin D_3, hormones, and/or antiandrogens.

30 Claims, No Drawings

102.

COMBINATIONS OF RETINOIDS AND MINOXIDIL-TYPE COMPOUNDS FOR HAIR GROWTH

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending application Ser. No. 136,525, filed Dec. 22, 1987, now abandoned, which in turn is a continuation of Ser. No. 463,146, filed Feb. 2, 1983, now abandoned, which in turn is a continuation-in-part of applications Ser. No. 235,169, filed Feb. 17, 1981; Ser. No. 318,607, filed Nov. 9, 1981; Ser. No. 386,730, filed Jun. 9, 1982; and Ser. No. 414,854, filed Sep. 3, 1982, all now abandoned. This application is also related to my co-pending application Ser. No. 283,649, filed concurrently herewith, entitled "Use Of Retinoids And Compositions Containing Same For Hair Growth".

FIELD OF INVENTION

This invention relates to the use of synergistic combinations with minoxidil (2,4,-diamino-6-piperidinopyrimidine-3-oxide) or certain of its derivatives or analogs in order to increase the rate of and stimulate growth of hair on mammalian skins, particularly human scalp hair to prolong the anagen phase of the hair cycle, to convert vellus hair to growth as terminal hair, and to treat certain types of alopecias.

BACKGROUND OF THE INVENTION

A normal characteristic of hair growth in mammals, including humans, is that in most cases, the rate of hair growth and the length of its growth cycle are reduced with age. Those phenomena are common to all mammals with rare exceptions, and they must be differentiated from true male pattern alopecia, which is caused by target organ sensitivity to androgens.

Several factors may influence the rate of hair growth. These factors include race, sex, age, geography, season of the year, nutrition and hormones. See Myers, R. J. and Hamilton, J. B. "Regeneration and rate of growth of hairs in man" *Ann. N.Y. Acad. Sci.* 53:562–568 (1951); Hamilton, J. B. "Age, sex and genetic factors in the relation of hair growth in man: A comparison of Caucasian and Japanese populations" *The Biology of Hair Growth* (Ed. Montagna, W. and Ellis, R. A.), Academic Press Inc., New York, pp. 400–433 (1958); Yano, S. "Rate of hair growth" *Hifu to Hinyo* 4:546–552 (1936); Maeda, I. "Study on the cuticula of hair: (III) Relation between the cuticula and rate of the growth of human hair" *Jyuzenkai-Zasski*, 43:1298–1304 (1938); Trotter, M. "The resistance of hair to certain supposed growth stimulants" *Arch. Dermatol. and Syphilol.* 7:93–98 (1923); Pinkus, F. "Zur Kenntnis der Lebensdauer der menschlichen terminal haare" *Z. Morphol. und Anthropol.* 24:256–269 (1924); Ono, M. "Studies on the hair growth of beard and scalp hair (1st report) Influencing factor in the rhythms of hair growth" *J. Physiol. Soc. Japan* 25:254–261 (1963).

Various preparations have heretofore been proposed for the treatment of male pattern baldness. It is also a matter of common knowledge, however, that none of the so-called "hair growth formulae" have proven to be very efficacious.

In contrast to most epithelial structures, the hair follicle does not grow continuously throughout its life, but passes through a cycle called the pilar cycle. The pilar cycle comprises essentially three phases—namely, the anagen or growth phase during which hair is produced, normally lasting about three to seven years; the catagen phase when growth stops and the follicle atrophies, lasting about three to four weeks; and the telogen phase, which is a rest period for the follicle during which the hair progressively separates and finally falls out, and normally lasting about three to four months. Normally 80 to 95 percent of the follicles are in the anagen phase, less than 1 percent being in the catagen phase, and the rest being in the telogen phase. Whereas the telogen phase hair is uniform in diameter with a slightly bulbous, non-pigmented root, the anagen phase hair has a large colored bulb at its root.

Alopecia results when the pilar cycle is disturbed, resulting in excessive hair loss. The most frequent phenomenon is a shortening of the hair growth phase due to cessation of cell proliferation. This results in an early onset of the catagen phase, and consequently a large number of hairs in the telogen phase during which the follicles are detached from the dermal papillae, and the hairs fall out. This shortening of the growth or anagen phase of the pilar cycle may have different origins, among which are very diverse pathological origins such as febrile conditions, mental stresses, hormonal problems (such as androgenetic alopecia due to male hormones) and secondary effects of drugs. Alopecia may also be due to age and to a slowing down of mitotic activity. This dysfunction of the biological mechanism of hair growth leading to alopecia may be regarded as a disease. While there are other causes of alopecia such as greasy or oily scalp due to seborrhea and the dandruff accompanying it, the present invention is not directed to treating these extraneous causes of alopecia, but rather to treating the organic dysfunction of the hair follicle.

German Patent No. 2758484 discloses certain chemical preparations for treatment of scalp to prevent baldness. These preparations contain bile compounds as the active ingredients and also include pro Vitamin A or tretinoin. The active ingredient is a product obtained from gall or a derivative thereof such as chenodeoxycholic acid, urodoxy cholic acid and their salts or derivatives.

Another patent is Olsen U.S. Pat. No. 4,140,229 citing the use of Vitamin A-containing crystal clear, transparent, aqueous, sprayable emulsions for reducing itching and flaking of common dandruff and seborrhea. As stated in its abstract, in some instances, the use of such emulsions reduced excessive falling hair. It does not purport to stimulate hair growth. It simply teaches a method of conditioning hair and scalp to effect relief from dandruff symptoms. The only pertinent example in Olson is discussed under Case History No. 3 of Example IV wherein the "Spray-on-Brush-in-Solution" contained Vitamin A palmitate and seven other ingredients. All that is disclosed is that "the daily loss of head hair was reduced to approximately 10 to 20."

Knight British patent specification No. 1,466,062 discloses a cosmetic composition containing tocopherol and retinoic acid as a cosmetic preparation which can be used on the skin or as a hair cleaning or hair dressing agent. This multi-purpose cosmetic composition allegedly prevents age spots, and is claimed to be good for clearing the scalp of dandruff. It appears that, during clearing of the scalp of dandruff with this composition, the scalp can become healthier, hair loss is reduced, and

hair growth and treatment. A specific treatment for androgenetic alopecia or male pattern alopecia is not suggested by this disclosure. The use of retinoids to alter the hair follicle growth rate or to prolong the anagen phase of the hair cycle is also not disclosed or discussed by Knight. Knight is claiming a cosmetic lotion for cleaning the scalp. Common dandruff and seborrhea or seborrheic dermatitis (seborrhea is the production of excess sebum and seborrheic dermatitis is an irritation of the scalp), as well as age spots, are the topic of this patent, and the composition used is a combination of two ingredients (Vitamin E and retinoic acid) in a cosmetic base.

There is a reference in the literature to the treatment of monilethrix using tretinoin (retinoic acid). Monilethrix is a vary rare genetic disease in which the hair shaft is defective and the hair is sparse and fragile. Topical application of retinoic acid improved the symptoms of this genetic defect. Hernandez-Perez, E. "Tretinoin therapy for monilethrix" *Archives of Dermatology* 109:575-576 (1974).

The use of retinoic acid in many disease conditions has been recently reviewed in the *Journal of the American Academy of Dermatology* by Haas and Arndt, "Selected therapeutic applications of topical tretinoin" 15:870-877 (1986). The review article in the May 1981 *Journal of the American Academy of Dermatology*, by Thomas, et al. also gives a list of the known uses of retinoic acid, but the treatment of alopecia or androgenetic alopecia is not listed.

There are no references of which I am aware for the use of retinoids in altering the rate of hair growth and treating alopecias, such as androgenetic alopecia. In fact, quite the opposite is the case, and the literature is full of references to hair loss caused by the toxic use of retinoids in high concentrations. References to hair loss caused by retinoids include W. Bollag and A. Matter, "From Vit A to Retinoids in Experimental and Clinical Oncology", p. 9-23, *Modulation of Cellular Interactions by Vitamin A and Derivatives, (Retinoids)* (Eds. Luigi M. DeLuca, Stanley S. Shapiro) Annals of New York Academy of Sciences, Vol. 359 (1981) and *Retinoids: Advances in Basic Research and Therapy* (Eds. C. E. Orfanos) Springer-Verlag (1981)—See articles "Aromatic Retinoids in Psoriasis", p. 165-173, S. Jablouska, et al.; and "Treatment of Severe Forms of Psoriasis and Retinoic Acid Derivatives", J. C. Gatti, et al., p. 185-191.

One compound, minoxidil, a potent anti-hypertensive compound, has been found to promote hair growth when applied topically to the scalp, as discussed in U.S. Pat. No. 4,139,619 and 4,596,812 to Chidsey et al. Minoxidil is recognized as being somewhat effective in producing new vellus hair growth and sparse terminal hair growth in a preselected group of subjects. However, its effect is far from satisfactory in most subjects.

BRIEF SUMMARY OF THE INVENTION

According to the invention it has been found that retinoids or mixtures thereof in combination with minoxidil and/or minoxidil-type compounds are synergistically effective in stimulating or increasing the rate at which hair grows on mammalian skin, prolonging the anagen phase of the hair cycle, converting vellus hair to terminal hair growth, and treating alopecias due to organic dysfunction of the hair follicle by topical application to the hair and hair follicles and to the skin adjacent thereto. Preparations such as lotions, creams, shampoos, and the like containing the aforementioned compounds as the active ingredients, can be applied topically to the skin, hair and/or follicles for this purpose. Oral administration of the retinoids may also be used. Other adjunctive compounds which may be included in the compositions of the invention include vitamins, such as Vitamin D_3, hormones, and antiandrogens. The invention also includes the topical or oral administration of retinoids to fur bearing animals or birds to increase the rate of hair growth and/or retard shedding or molting.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Retinoids have been shown to cause elevated DNA synthesis in keratinocytes in cell culture. Retinoids can also be shown to increase the turn-over time of epidermal cells in cell culture experiments as well as in vivo experiments with human subjects. As disclosed in my copending application Ser. No. 283,649, the present inventor has discovered that the cells of the hair follicle, particularly the keratinocytes, can be stimulated by retinoids. When tested experimentally, the retinoids caused the cells of the dermal papillae and the keratinocytes, as well as cells of the root sheath, to incorporate more tritiated thymidine into DNA and to reproduce at a more rapid rate than untreated cells from other hair follicles. This stimulation by the retinoid compounds ultimately causes the entire hair follicle to become more activated and the mitotic index, as measured by thymidine-H^3 incorporation into DNA, to rise. Therefore, the individual scalp hairs can be shown to grow at an increased rate, and the anagen phase is prolonged.

A major problem in influencing alopecia is to revascularize the area of alopecia and initiate the primary new hair growth. Retinoic acid and its derivatives and the other retinoid compounds have been shown to give excellent percutaneous absoprtion and to be very active on the keratinizing cells of the skin, including the hair follicle. However, it is difficult for retinoids alone to revascularize the area of the pilosebaceous apparatus.

Studies have shown that minoxidil, a potent antihypertensive medication and peripheral vasodilator, can increase the rate of hair growth on the body when taken systemically, particularly in areas of the limbs and facial areas, possibly due to vasodilatory properties. Further studies have suggested that minoxidil may be effective in initiating and promoting vellus hair growth on the scalp of individuals with alopecia. However, minoxidil may not able to sustain the growth of terminal hairs from vellus hairs on the scalp. In the majority of subjects with alopecia, terminal hair growth on the scalp may not be initiated or sustained by the topical application of minoxidil nor by its systemic administration.

Minoxidil has been shown to prolong the life of keratinocytes in culture and extend the time after confluence that cells can be subcultured. These data suggest that the mechanism by which minoxidil exerts its effect is that the drug reduces the rate at which cells are lost from the germinative pool and hence slows senescence. See J. Kubilus et al., "Effect of Minoxidil on Pre- and Postconfluent Keratinocytes," *Journal of the American Academy of Dermatology*, 16:648 (1987). Vascular effects alone do not appear to be a sufficient stimulus for hair growth, particularly in an area affected by alopecia. As described in my copending application Ser. No. 283,649, the disclosure of which is incorporated herein by reference, retinoids can stimulate and increase the

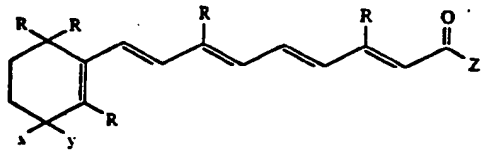
hair growth in both males and females can prolong the anagen phase of the hair cycle, as well as converting vellus to terminal follicles. The mechanism of action of the retinoid compounds is believed to be through the initiation and activation of increased cell turnover and cell differentiation, i.e., compounds which of themselves can initiate the differentiation of cells of the pilosebaceous apparatus which eventually form the hair follicle and become terminal hairs.

Because of the advanced state of scalp thinning and atrophy of the pilary portion of the pilosebaceous apparatus, it is difficult to initiate hair growth from areas of advanced male pattern alopecia. Retinoid compounds sustain and promote hair growth in areas where hair is present to some extent.

The present invention combines the use of retinoid compounds with minoxidil, or its analogs or derivatives or minoxidil-type compounds (hereinafter collectively referred to simply as "minoxidil"). The stimulatory actions of both compounds can synergistically promote each others' effect. Retinoids can initiate cell growth and differentiation (not initiated by minoxidil), and minoxidil can promote the vasodilatory and mitogenic action not obtained with the retinoids. While neither compound alone may have profound effects on advanced alopecias, in combination the compounds are very effective as promoters of new hair growth in areas of alopecia.

The net result of application of minoxidil and retinoids is initiation and production of new hair growth and conversion of vellus to terminal hair growth, i.e., the increase in size from a vellus to a terminal hair and the continued and more prolonged maintenance of the hair in the anagen phase. As noted previously, this effect is obtained not merely as the addition of two compounds, but as synergism, i.e., the combination of these substances in the present invention produces an effect which cannot be produced by either compound separately under conditions of its use and, therefore, represents a major advance in the treatment of alopecia.

Suitable retinoid active ingredients for use in this invention include derivatives of retinoic acid (Vitamin A acid or tretinoin) which may be represented by the following formulae:

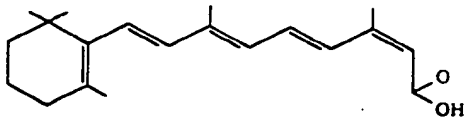


wherein R is hydrogen or a lower alkyl group, X is individually hydrogen and Y is individually hydrogen or a hydroxy group, or X and Y together form oxo, and Z is alkoxy, amide, alkylamide, hydroxy, nitro, or other suitable terminal groups. Also included by the above formula are pharmaceutically accepted salts thereof.

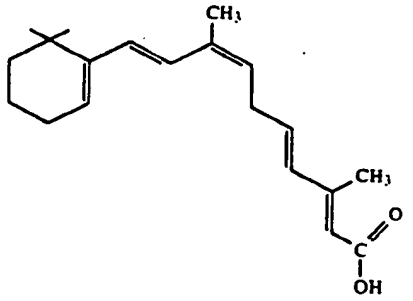
Further, the basic formula may include the dehydro, dihydro, or anhydro forms, such as the 7,8-dehydro and 5,6-dihydro forms, of retinoic acid as well as all of the stereoisomeric forms thereof, such as the 9-cis; 9,13-dicis; 13-cis; 11-cis; 11,13-dicis; etc. Examples are shown as follows:

13-cis-retinoic acid

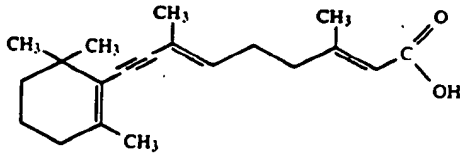
-continued



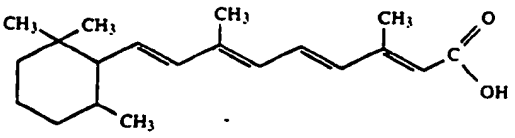
9-cis-retinoic acid



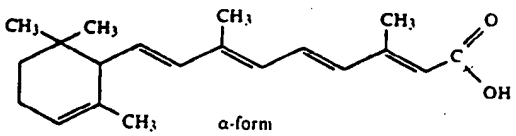
7,8-dehydro-retinoic acid



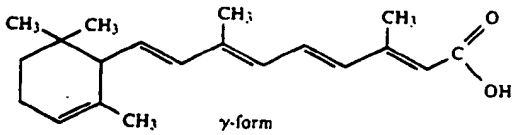
5,6-dihydro-retinoic acid



The anhydro forms may be represented by the following compounds:

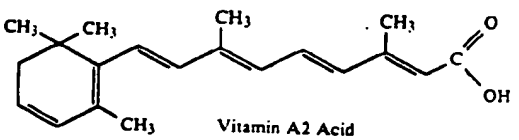


α-form

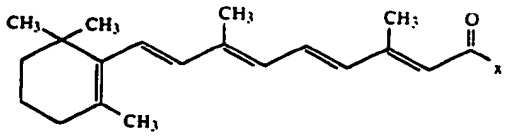


γ-form

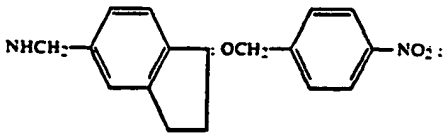
Suitable retinoid analogs and derivatives useful in the invention have the following general formulae wherein the side chain, the ring, or both, may be altered:

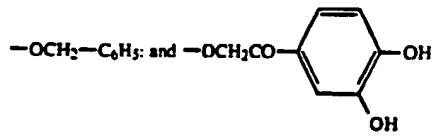


Vitamin A2 Acid



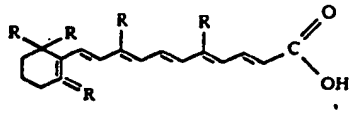
wherein X is a member selected from the group consisting of: $-OHCH_2CONH_2$: mixed $-OCH_2CH(OH)CH_3$ and $-OCH(CH_3)CH_2OH$; $-OCH$; as well as



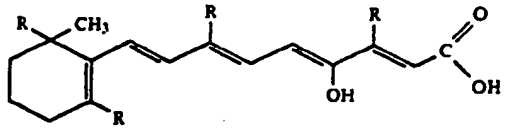


These compounds as well as other alkoxy and amide compounds can be active as they can be hydrolyzed to retinoic acid and other active compounds in the body. However, their activity may not be as direct as all-trans retinoic acid.

Other suitable retinoid compounds useful in the invention include α-hydroxy retinoic acid represented by the formula:

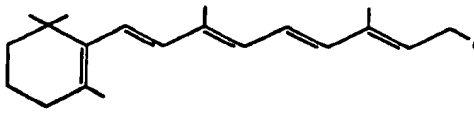


and the C_{22}-analog of retinoic acid represented by the following general formula:

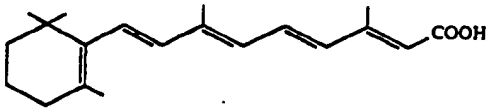


wherein R in both of the above formulae are lower alkyl radicals, preferably methyl groups.

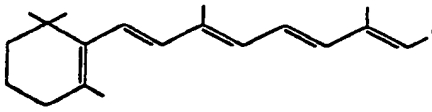
Other structurally modified retinoids which, to some degree, exhibit the activity of retinoic acid for hair growth purposes can be presented by the following general formulae:



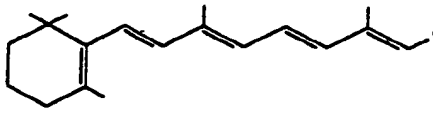
retinyl acetate



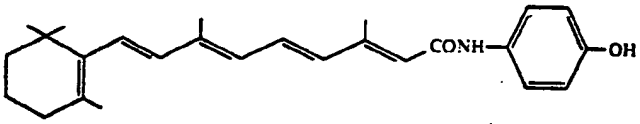
(all-E)-retinoic acid



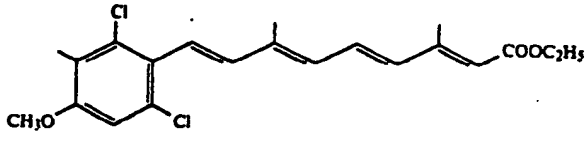
Retinoic Acid Ethyl Amide

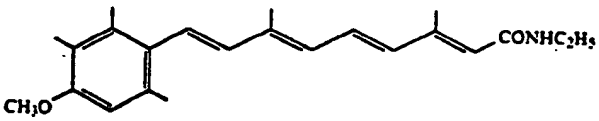


Retinoic Acid 2-Hydroxyethyl Amide

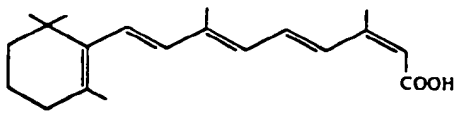


Retinoic Acid p-Hydroxyphenyl Amide

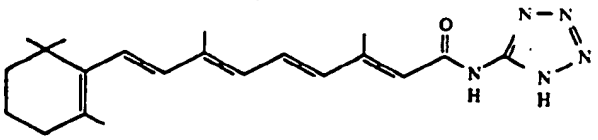




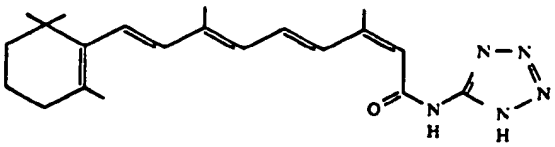
-continued



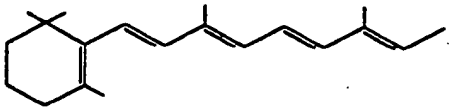
(13Z)-retinoic acid



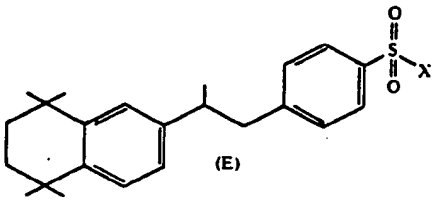
35689-BASF



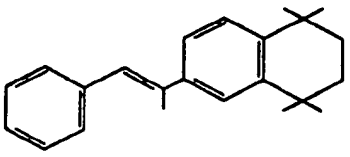
37175-BASF



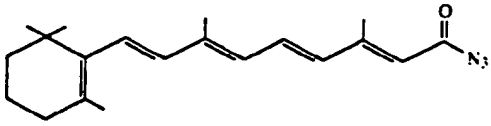
35739-BASF



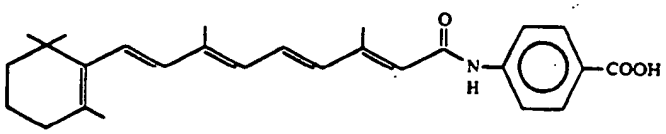
(E)



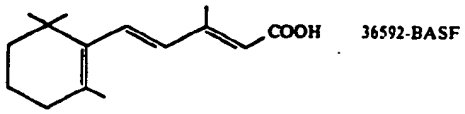
arotinoid



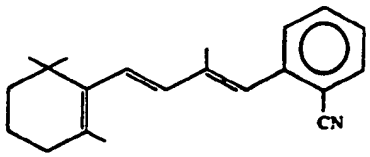
36591-BASF



35681-BASF

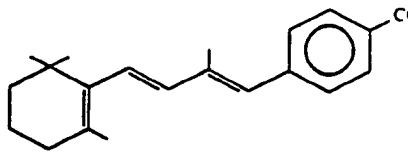


36592-BASF

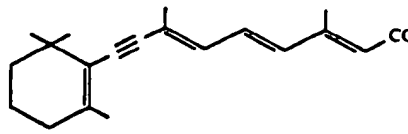


36663-BASF

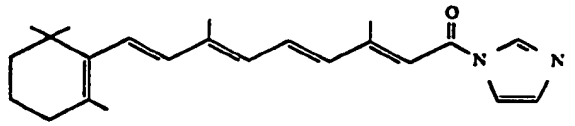
-continued



37400-BASF

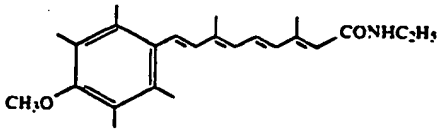


35684-BASF

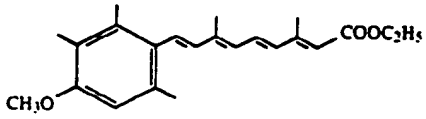


(all-E)-N-(1-imidozolyl)-retinamide

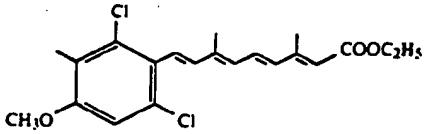
Still other useful analogs and derivatives of retinoic acid and retinoids include the following compounds:



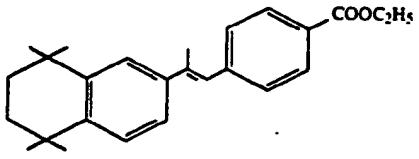
Trimethylmethoxphyenyl (TMMP) analog of retinoic acid ethylamide (Motretin)



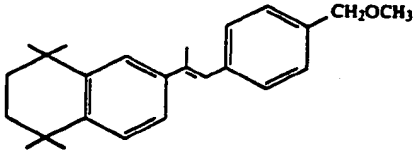
Trimethyimelhoxphenyl (TMMP) analog of retinoic acid ethyl ester (Etretinate)



Dichloromethylmethoxphenyl (DCMMP) analog of retinoic acid ethylester

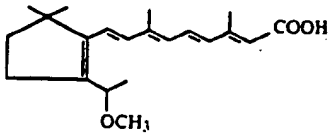


Arotinoid ethyl ester

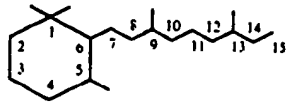


Arotinoid

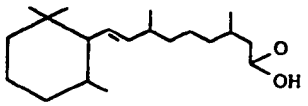
-continued



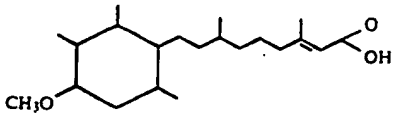
1-Methoxyethyl-cyclopentenyl analog of retinoic acid



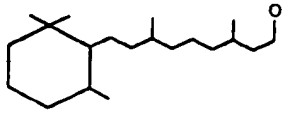
Axerophihene



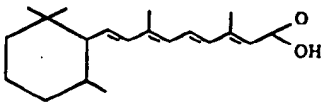
13-cis-Retinoic acid



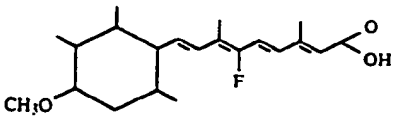
Trimethylmethoxyphenyl



Retinal

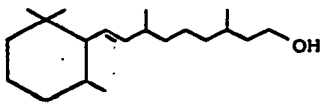


β-all-trans-Retinoic acid (RA)

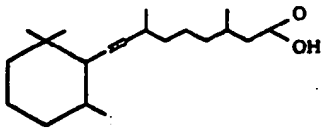


10-Flouro-TMMP anaolog of RA

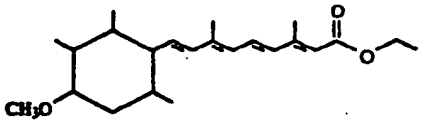
-continued



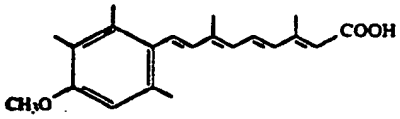
Retinol

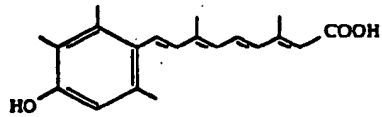


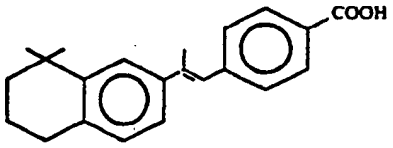
7,8-Dehydro analog or RA

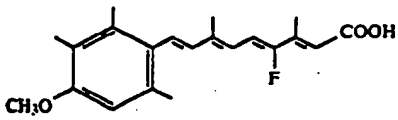


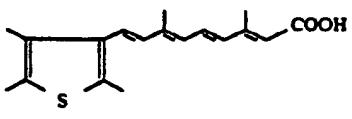
TMMP analog of ethyl retinoate

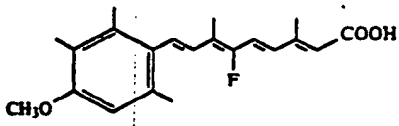


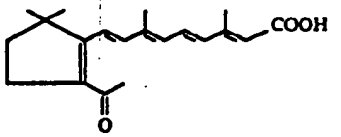


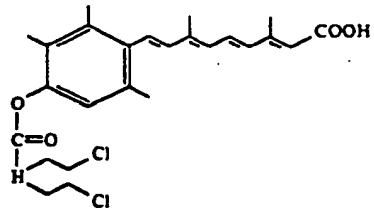


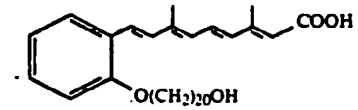


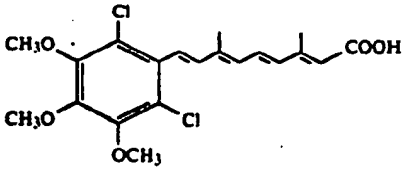


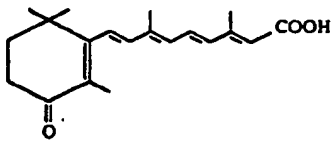


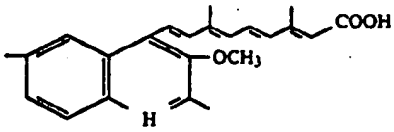


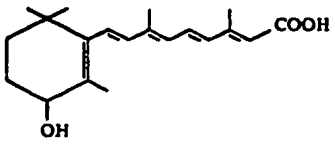


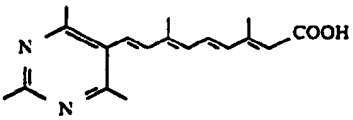


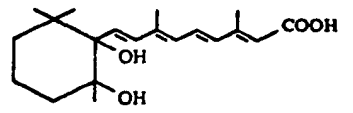


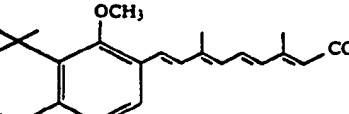


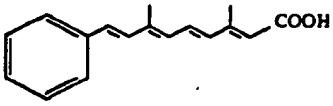




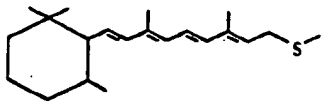




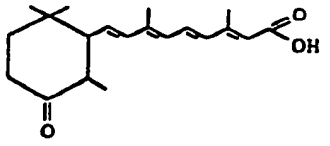




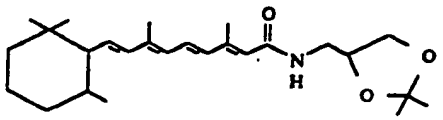
-continued



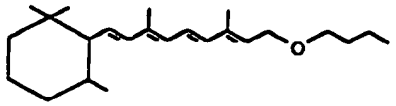
Retinyl methylthioether



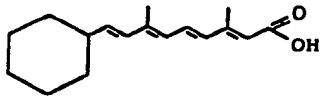
4-Oxo analog of RA



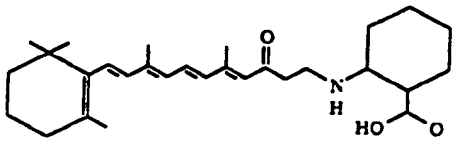
M-Methyl-dimethyldioxolan-retinomide



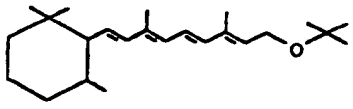
Retinyl n-butyl ether

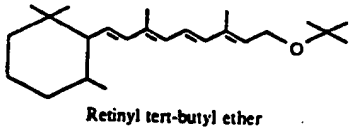


Phenyl analog of RA

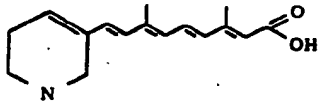


N-(O-Carboxyphenyl)-retinomide

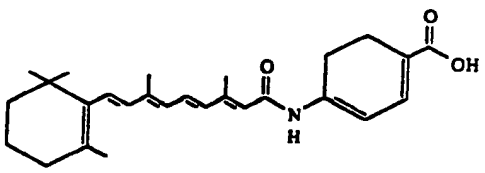




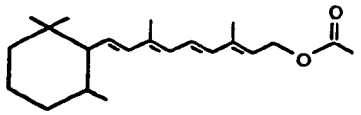
Retinyl tert-butyl ether



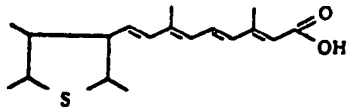
Pyridyl analog of RA



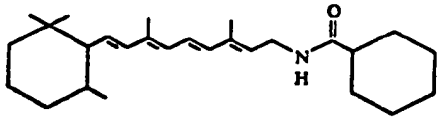
N-(p-Carboxyphenyl)-retinomide



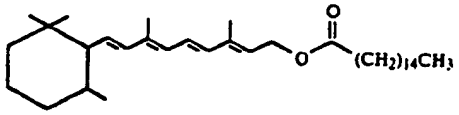
Retinyl acetate



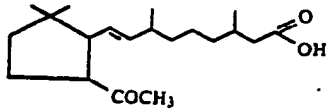
Trimethylthiophene(TMT) analog of RA



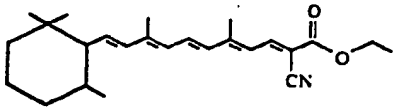
N-Benzoyl-retinylamine



Retinyl palmitate

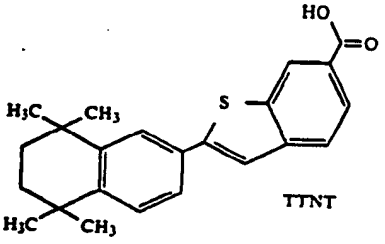


Dimethylacetyl cyclo-



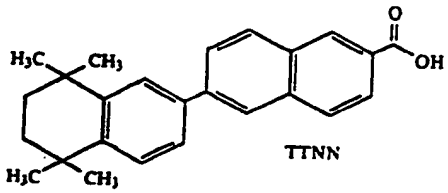
Retinylidene ethylcyano-

BENZOTHIOPHENE



TTNT

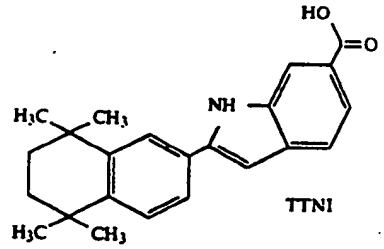
NAPHTHALENE



TTNN

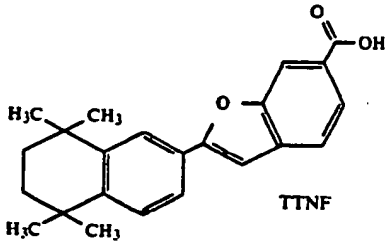
INDOLE

-continued

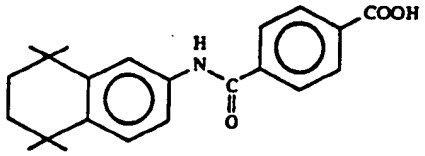


TTNI

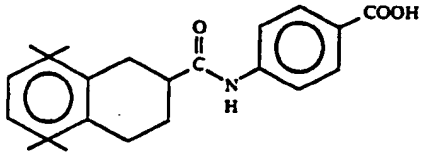
BENZOFURAN



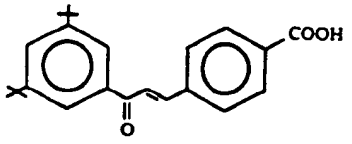
TTNF



Am-80



Am-580



Ch-55

Also included within the foregoing compounds are any halogenated compounds or ether, amide, modified rings, dehydro, dihydro, isomer or analog forms of said compounds.

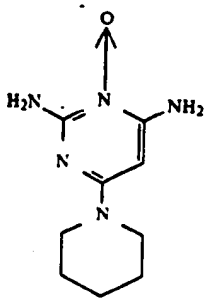
The retinoid compounds useful in the present invention are believed to have the common characteristic of binding to the retinoid cell receptors and thereby stimulating the hair follicle cell proliferation.

Retinoids have been defined narrowly as comprising simply Vitamin A (retinol) and its derivatives such as Vitamin A aldehyde (retinal), Vitamin A acid (retinoic acid), comprising the so-called natural retinoids. Retinol and its esters have been used previously in hair preparations to prevent hair loss, but not to increase or stimulate hair growth in cases of alopecias.

Subsequent research has resulted in a much larger class of chemical compounds that are termed retinoids due to their biological similarity to Vitamin A and its derivatives. Compounds useful in the present invention include natural forms of Vitamin A, Vitamin A acid and its isomers, Vitamin A aldehyde and/or synthetic analogs of Vitamin A acid which possess the biological activity of Vitamin A acid in the hair follicle. Accordingly, as used herein for purposes of the present invention, the term "retinoid" will be understood to include any compound which fits the foregoing chemical and/or biological definitions.

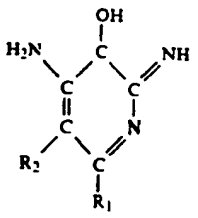
That is, the definition of a retinoid intended in this invention is a substance that can elicit a specific biological response by binding to and activating a specific receptor or set of receptors for retinoids. Therefore, any Vitamin A type compound, whether defined by the classic description of a particular subset of diterpenoid, polyene substances or a new type of synthetic ligand (neither diterpenoid nor polyene) which can have a better molecular fit to the retinoid receptors (cytosolic retinoic acid binding proteins), should be considered in this definition. The biological response of the target cells for retinoids should be defined as any compound (retinoid) which is capable of stimulating the hair follicle cells to differentiate or to turnover more rapidly. This covers compounds traditionally related to retinoids and it also covers compounds which are not diterpenoid types. The ring, the side chain, the terminal group or all of these can be altered. This definition would include even newer retinoids which do not fit the older Vitamin A-type concept but which can be shown to bind to the retinoid receptor proteins specific for retinoic acid (CRABP) within cells of the follicular epithelium. Examples of such newer retinoids include, inter alia, TTNT, TTNN, TTNI, TTNF, Am-80, Am-580 and Ch-55, which are shown above.

Minoxidil (2,4-diamino-6-piperidinopyrimidine-3-oxide) is represented by the following formula:

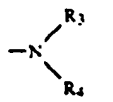


In addition to minoxidil, its active derivatives and analogs can also be used. These active derivatives and analogs are described, for example, in U.S. Pat. Nos. 5,910,928; 3,637,697; 3,461,461; 4,139,619; and 4,596,812, the descriptions of which are fully incorporated herein by reference.

Among the active derivatives and analogs of minoxidil described in these patents are compounds of the formula:



where R_1 is a moiety selected from the group consisting of moieties of the formula



wherein R_3 and R_4 are selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, lower arylalkyl, and lower cycloalkyl, and taken together R_3 and R_4 may be a heterocyclic moiety selected from the group consisting of aziridinyl, acetidinyl, pyrrolidinyl, piperidino, hexahydroazepinyl, heptamethylenimino, octamethylenimino, morpholino, and 4-lower alkylpiperazinyl, each of said heterocyclic moieties having attached as substituents on the carbon atoms 0–3 lower alkyl groups, hydroxy or akloxy, and wherein R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, lower alkoxyalkyl, lower cycloalkyl, lower aryl, lower aralkyl, lower alkaryl, lower alkaralkyl, lower alkoxyaralkyl, and lower haloaralkyl, and the pharmacologically acceptable acid addition salts thereof.

See also *J. Heterocyclic Chem.*, 15:1529 (1978) by John M. McCall, et al., the disclosure of which is additionally incorporated herein by reference.

Included as minoxidil analogs are those described in the following U.S. Pat. Nos.: 4,287,338; 4,220,772; 3,464,987; 4,316,901; 3,270,015; 3,270,014; 3,382,248; 3,461,461; 4,080,500; and 3,973,016, the disclosures of which are incorporated herein by reference. Other suitable minoxidil-type compounds include minoxidil glucuronides disclosed in published European application 0242967A1 and the substituted pyrimidine compounds disclosed in my published PCT patent applications WO86/00616 and WO/8504577.

A major problem in influencing hair growth is obtaining good percutaneous absorption of the active compounds. The retinoid compounds described herein cause excellent percutaneous absorption of themselves and other compounds used in combination therewith, and are very active on the keratinizing cells of the skin, including the hair follicles.

Accurate measurement of hair growth to substantiate the results of the testing is often a problem. A microcapillary method which gives excellent results and can be used to measure the rate of hair growth was devised by M. Saitoh, et al., *Advances in Biology of the Skin*, vol. 6, p. 467 (1968) and utilizes microcapillary tubes which are graduated using 0.2 mm graduations. A less time-consuming magnification method which also yields good results involves shaving off of the hairs for examination and measurement.

The pharmaceutical, cosmetic or veterinary preparations of the present invention can be prepared by conventional techniques for the preparation of lotions, creams, conditioners or shampoos for the scalp or veterinary preparations for pelts. Though not as preferred, included also are preparations which can be administered orally and compounds which can be added to animal foods.

In addition to the active combinations of retinoids and minoxidil-type compounds of this invention, the various preparations can contain any conventional pharmaceutically acceptable or cosmetically acceptable inert or pharmacodynamically active additives or carriers. For example, the lotions may be prepared using various forms of alcohols or other solubilizers such as glycols or esters. The conditioners may contain the normally acceptable, comm[illegible] pounds such as ceryl alcohol, c[illegible] ins, hydrolyzed animal protein, [illegible] amodimethicone, paraffin, mineral oil, si[illegible]

The topical compounds may also contain adjunctive compounds, such as oils, including essential fatty acids; vitamins or their derivatives; hormones (natural or synthetic), including progesterones, estrogens including estradiols, thyroids, and polypeptide hormones; and antiandrogens, including but not limited to cyproterone acetate, cyoctol, secosteroids, flutamide or spironolactone, and particularly nonsteroidal antiandrogens such as the decahydro-7H-benz(E)-inden-7-ones described in U.S. Pat. No. 4,466,971. Androgens are known to cause alopecia in genetically programmed individuals, and antiandrogens prevent the effect of the androgen on the nucleus of the hair follicle cell. Therefore, any substance which can prevent the androgen from acting on the nucleus of the cell is considered an antiandrogen.

Examples of the active-type Vitamin D_3 which can be used in combination with the retinoids of this invention include the following types which are not meant to be limiting: 1-hydroxycholecalciferol; 1,25-dihydroxycholecalciferol (commercially available as ROCALTROL); and 1,24-dihydroxycholecalciferol. Vitamin D3 is generally administered at a rate of about 0.001 to 0.3 μg/gm. Vitamin D_3 type compounds have recently been shown to regulate cell differentiation and to promote the differentiation of the keratinocyte. Vitamin D compounds are also important in calcium regulation. These compounds may assist in the conversion of vellus to terminal hairs.

The topically applied lotions, creams, conditioners, or other formulations containing the retinoid will vary according to the standard art with regard to the amounts of other hydrophilic and hydrophobic ingredients, including emulsifiers, so that either an oily, semi-oily or oil-free product may be obtained. The shampoos may contain any of the conventionally used detergents or soaps and any other compounds used by those familiar with the art. Oil-based shampoos are included in these formulations.

The oral preparations may be tablets, liquids, capsules, etc. The pharmaceutically acceptable substances commonly used as preservatives, stabilizers, moisture retainers, emulsifiers, etc., can be present in these preparations. Conventionally acceptable antioxidants such as tocopherols, N-methyl 2-tocopheramine, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), can be incorporated also in the preparations described herein.

Retinoids and minoxidil are administered in effective amounts which vary with the route of administration and the requirements of the subjects. The topical treatments may consist of lotions, creams, conditioners, shampoos, oil treatments, etc., with about 0.001 to 2% by weight of all-trans retinoic acid or derivatives, or other retinoids, as the preferred dosages in the described compositions and with dosages of about 0.01% to 30% minoxidil in suitable vehicles for topical use. Arotinoids may be effectively used at doses as low as 0.00001% by weight or even lower. Oral dosages for minoxidil should not exceed 10 mg/day total dose, and for retinoic acid 1 mg/kg body weight/day is a maximal dose which will eventually cause toxicity and with chronic treatment will probably cause the hair to fall

enhancer!

out. The [illegible] mg/kg body weight should be a [illegible] dose.

To examine the specific action of the retinoic acid and minoxidil in increasing the rate of hair growth, several types of experiments were performed. The microcapillary method was used in each case to measure the rate of hair growth. Other methods of dye staining and hair growth measurements were also undertaken. In addition, the method of Ebling, et al., *Journal of Investigative Dermatology*, November 1981, was used to study the conversion of vellus to terminal hairs by microscopy.

The effectiveness of the active ingredients of this invention for increasing the rate of or stimulating hair growth will now be illustrated by the following examples. These examples, however, are merely representative and should not be construed so as to limit the scope of the invention.

EXAMPLE I

A topical lotion containing 0.1 percent by weight of all-trans retinoic acid and 3% by weight minoxidil was included in the preparation to be tested. As the vehicle, 5 weight percent propylene glycol, 1 mg per 100 ml butylated hydroxytoluene (BHT), and 95 weight percent ethanol were mixed in a beaker for several minutes at ambient conditions to obtain a homogeneous preparation in which the retinoid was dissolved. This lotion was labelled A and was the active drug preparation. A similar lotion was prepared by the foregoing procedure containing all the aforementioned ingredients, but omitting the retinoid and minoxidil. This preparation was labelled B and was the placebo lotion.

Volunteer subjects had a 3 to 4 cm diameter area of scalp hair bleached at the scalp end. The subjects were asked to apply the (placebo) lotion B, 2 times a day, to the scalp for varying periods of time from 10 to 30 days. The rate of hair growth in each individual was determined using measurements taken with either microcapillary measuring equipment or by magnification measurements. The rate of new hair growth from the scalp end to the bleached area was recorded every three to six days. The data is expressed as control rate of hair growth in mm of growth per day.

At the end of the placebo treatment, anagen/telogen ratios were determined by the following standard method of Orentreich, N. and Berger, R. A. "Selenium disulfide shampoo", *Arch. Derm.* 90:76–80 (1964): Hair plucking Was done from the areas treated before and after treatment. A large surgical-needle-holding clamp, with jaws covered with a smooth layer of rubber was used. Twenty to fifty hairs were grasped at one time, approximately 1.0 cm above the scalp surface, and epilated with a single forceful pull. The hair roots and lower portion of the shafts were then cut off into shallow plastic Petri dishes, previously gridded into 1 cm squares, moistened with tap water, and examined and counted by transmitted light with a low-power dissecting microscope. The roots could also be stained to help in the interpretation.

Following the placebo treatment, the subjects were given lotion A (containing the active drug) or lotion B and asked to apply the lotion in the same manner in which they had applied the previous (placebo) lotion. The same procedure was followed for measuring hair growth, namely every three to six days the subject returned for measurements to determine rate of hair growth. At the end of the active drug treatment period, subjects again had anagen/telogen ratios determined. Neither the subjects nor the observers were told which lotion A or B was the active preparation until after the data were analyzed.

Before treatment and at monthly intervals thereafter, a circle 1 inch in diameter was drawn over the balding spot of the vertex with a skin marker and a template. The center of the circle was located by a three-point measurement, using the midpoint between the ears and a fixed distance from the base of the nose. These measurements were recorded at each visit. With the aid of a magnifying lens, the hairs in the 1-inch diameter circle were counted and typed as vellus hairs, indeterminate hairs, or terminal hairs. Nonpigmented short hairs were defined as vellus; pigmented hairs ranging from thin and short to slightly longer and thicker were defined as indeterminate. Hairs of the same color and bore diameter as those in adjacent nonbalding areas were classified as terminal. The count was repeated several times and the average used as the final count. The number of vellus and terminal hairs were compared before, during and at the final visit, and calculated as percent conversions from vellus to terminal.

In Table I are described the results of studies using male and female subjects. The all-trans retinoic acid and minoxidil in lotion form was applied topically or as described in Table I, and hair growth rates were assessed along with conversion of vellus to terminal hair.

TABLE I

Lotion Containing All-Trans Retinoic Acid 0.1% and Minoxidil 3%

Subject		Dosage	Form of	Treatment Time	Rate of Growth (mm/day)		% Conversion
Sex	Age	(ml/day)	Dosage	(Months)	Control (Lotion B)	Treatment (Lotion A)	Vellus to Terminal
M	37	10	Topical	2	0.23	0.30	11%
M	62	10	Topical	2	0.21	0.29	22%
M	38	10	Topical	2	0.35	0.42	35%
F	43	10	Topical	2	0.37	0.39	13%
F	38	10	Topical	2	0.31	0.35	18%
F	64	10	Topical	2	0.24	0.29	17%

COMPARATIVE STUDY

A group of twenty normotensive subjects, twenty to sixty-four years of age and clinically diagnosed as suffering from androgenetic alopecia, were entered into a combined study in which twelve subjects received 0.025% topical tretinoin solution with the vehicle as discussed above (95% ethanol, 5% propylene glycol and 1 mg BHT per 100 ml), 36 subjects received a combination of 0.025% tretinoin and 0.5% minoxidil solution, five subjects received the vehicle alone as a pla-

cebo, three subjects received 0.5% minoxidil solution alone, according to the following protocol. Food coloring was added to the placebo and minoxidil solutions to match the color of the retinoic acid.

The subjects were instructed to apply 1 ml of the solution twice daily by dropper to the affected scalp area (a circular area of baldness, 1 inch in diameter). The subjects were advised to wear a cap for protection from the sun or to refrain from excessive sun exposure, and to avoid trauma to the scalp (i.e., vigorous scalp scrubbing or brushing). Blood pressure, serum chemistry tests, complete blood counts, weight, pulse and electrocardiogram were performed before treatment and at repeated intervals for each patient; skin irritation was assessed during each follow-up visit; photographs were taken before and during treatment to evaluate hair growth; and hair counts were performed initially, at monthly intervals, or at follow-up visits.

After analysis of the data, the subjects were placed into one of three designated response groups, with percent increase in number of terminal hairs (defined as thick, pigmented hairs, comparable to those on the subjects' posterior scalp) being the primary criterion for placement. Participants in the "good" response group (Group A) experienced greater than 46% increase in the number of hairs in the target area after treatment; individuals in the "moderate" response group (Group B) had a post-therapy terminal hair increase between 21% and 45%; while participants in the "no response" group (Group C) experienced increases below 20%. The mean amount of time for subject participation was 10, 8 and 9 months, respectively, for Groups A, B and C, and the results are indicated in Table II below.

TABLE II

Treatment	No. of Patients	Response: Good (Group A)	Moderate (Group B)	None (Group C)
Placebo	5	0	0	5 (100%)
Minoxidil	3	0	0	3 (100%)
Tretinoin	12	2 (16%)	5 (42%)	5 (42%)
Combination	36	16 (44%)	8 (22%)	12 (33%)

In 56 subjects, 48 of whom were receiving tretinoin or the combination formulation, positive responses were documented in more than half of the subjects, usually within 18 months. The five patients receiving placebo demonstrated no significant hair growth response. Three patients receiving the 0.5% minoxidil solution also showed no meaningful results. Of the 5 men who received tretinoin only, two experienced some hair growth after treatment, although the hairs were mostly of the lanugo (vellus) type.

However, surprisingly, of the patients treated with the combination solution (0.5% minoxidil and 0.025% tretinoin), 66% responded positively, with 44% placed in the good response group and 22% in the moderate response group. These data suggest that there may be a synergism between minoxidil and tretinoin when the substances are combined and used topically. While neither compound alone appears to have profound effects on advanced alopecias, in combination the compounds may be more effective as promoters of new hair growth in individuals with alopecia.

While this study used only 0.5% minoxidil in combination with retinoic acid, other studies report that 2% to 5% minoxidil concentrations cause a cosmetically visible hair regrowth in 30% to 40% of subjects. The above results show that low concentrations of minoxidil (only 1/4 to 1/10 the topical minoxidil concentrations previously reported) are effective when used in combination with tretinoin, suggesting that mixtures of minoxidil and retinoids may be more effective in the treatment of alopecia than is minoxidil alone.

More details of the above study, including photographs of the patients, may be found in Bazzano et al., "Topical Tretinoin for Hair Growth Promotion," *Journal of the American Academy of Dermatology*, 15:4, Pages 880–883 and 889–893 (October 1986).

The following Examples illustrate forms of topical application of compositions of the present invention. The methods of administration may vary by lotion, cream, ointment, pill, supplement to animal food, coating for seeds, etc. These Examples are only meant to be illustrative, and do not limit the mode of administration nor the ingredients which can be admixed to the present invention, nor the amounts which may be used.

FORMULATION EXAMPLE I

Lotion Formulation for the Topical Administration

Ingredients	Weight %
All-trans retinoic acid or 13-cis retinoic acid	0.01 to 0.1
Minoxidil	0.5 to 5.0
Ethanol	q.s. to 100.0
Propylene glycol	5.0 to 50.0
Butylated hydroxytoluene (BHT)	0.1
Distilled water	up to 10.0

FORMULATION EXAMPLE II

Cream Conditioner for Topical Administration

Ingredients	Weight %
All-trans retinoic acid or 13-cis retinoic acid	1.0
Minoxidil	10.0
Distilled water	q.s. to 100.0
Cetrimonium Chloride	5.0
Cetyl alcohol	4.0
Ethanol	4.0
Butylated hydroxytoluene	1.0
Hydrolized animal protein	0.5
Methylparaben, propylparaben	0.1
Stabilizer	0.1

In this example, a higher concentration of active ingredient was used since the conditioner is rinsed out shortly after application.

FORMULATION EXAMPLE III

Hydrophilic Ointment for Topical Administration

All-trans retinoic acid (0.01 to 0.1 gram) and 1-10 grams of minoxidil are dissolved in 100 ml of acetone, and the solution is then admixed with 900 g of USP grade hydrophilic ointment to a uniform consistency; one gram of butylated hydroxytoluene is added. A water washable cream ointment is thus prepared.

FORMULATION EXAMPLE IV

Tablets for Oral Administration

Ingredients	Amounts
Minoxidil	10 mg.
All-trans retinoic acid or	25 mg.

-continued

Ingredients	Amounts
13-cis retinoic acid	
Lactose	52 mg.
Cornstarch	20 mg.
Microcystalline cellulose	40 mg.
Talc	2.5 mg.
Magnesium stearate	0.5 mg.

The active ingredients are mixed with lactose and granulated using a cornstarch paste. The remainder of the above adjuvants are then admixed therein, and the mass is tableted. The tablets are then coated with a water-soluble or water-swellable lacquer. Liquids, syrups or other formulations can be made consistent with pharmaceutical art.

The retinoid/minoxidil combinations of the invention may also be used in veterinary preparations or feeds to increase the rate of growth of fur (pelt) in certain fur bearing animals and to retard shedding and molting.

In fur bearing animals, the rate of fur growth, length of hair, thickness of hair and molting season are controlled by many factors including season, light (wavelength) periodicity, temperature, hormonal factors and nutrition. Controlling all of these variables is impossible. However, animals were selected and areas over the hind quarters were shaved in 2 inch diameter circular areas. In some of the animals the areas were treated topically with all-trans retinoic acid, and in other animals the retinoid was administered orally in animal chow. Some of the animals served as their own controls, using treated and non-treated areas.

In fur bearing animals, the guard hairs and the pile hairs differ in thickness, length and growth rate. In the rabbits studied, the guard hairs averaged 34 mm and the pile hairs 30 mm in length. The effect of topical application of all-trans retinoic acid was to increase the rate of new hair growth. An effect on the non-shaved fur bearing areas treated with topical all-trans retinoic acid in lotion form, was a decrease in the shedding or molting of fur. The mean rate of hair (fur) growth from treated shaved areas was 0.3 mm per day for 3 rabbits (mean) while in non-treatment shaved areas it averaged 0.2 mm per day (mean of 3 rabbits).

The effect could also be demonstrated in domestic cats and dogs; the same type of experimental procedures were used. The most striking effect in long haired dogs and cats was the retardation of molting or hair shedding. Long haired dogs and cats tended to retain more hair in the anagen phase and there was approximately 50% less shedding during the treatment periods. Both methods of administration were satisfactory. Either topical lotion or cream treatment or systemic treatment by inclusion in animal chow was satisfactory. The daily dosage for animals was 20 mg per kilogram animal chow or 10 to 15 mg applied topically.

Commerically important fur bearing animals were also used for experimentation. Two male minks were closely clipped over the back hind quarters. The animals were treated on one hind quarter and the other was used as the control. The microcapillary method for measuring hair growth was used for these studies. The animals were treated by two different methods. The animals were either fed the retinoid in their chow or they were administered the retinoid topically. The daily dose was 20 mg per kg animal chow or 5 mg per day applied topically. The results of these experiments showed that the rate of growth of new pelt was increased approximately 30% by the retinoid treatment.

Experiments using birds (canaries and parakeets) showed that inclusion of the all-trans retinoic acid or the ethyl ester of all-trans retinoic acid in bird food at a dosage of 30 mg per kilogram bird seed retarded the molting process.

The present invention may be embodied in other specific forms without departing from the spirit or the central attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification as indicating the scope of the invention.

I claim:

1. A composition for treating alopecia comprising a retinoid and a minoxidil compound, said compound being present in an amount of about 0.01 to 30 percent by weight and said retinoid being present in an amount of about 0.001 to 2 percent by weight in said composition.

2. A composition according to claim 1 wherein said retinoid is Vitamin A acid.

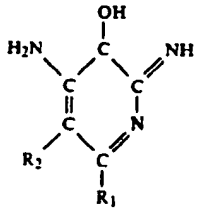
3. A composition according to claim 1 wherein said compound is minoxidil (2,4-diamino-6-piperidinopyrimidine-3-oxide).

4. A composition according to claim 3 wherein said retinoid is Vitamin A acid.

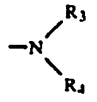
5. A composition according to claim 1 wherein said composition also includes a pharmaceutically effective vehicle for said compound and said retinoid.

6. A composition according to claim 5 wherein said vehicle comprises ethanol and propylene glycol.

7. A composition according to claim 1 wherein said compound has the formula:



wherein R_1 is a moiety selected from the group consisting of moieties of the formula



wherein R_3 and R_4 are selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, lower aralkyl, and lower cycloalkyl, and taken together R_3 and R_4, may be a heterocyclic moiety selected from the group consisting of aziridinyl, azetidinyl, pyrrolidinyl, piperidino, hexahydroazepinyl, heptamethylenimino, octamethylenimino, morpholino, and 4-lower alkylpiperazinyl, each of said heterocyclic moieties having attached as substituents on the carbon atoms 0–3 lower alkyl groups, hydroxy or alkoxy, and wherein R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, lower alkoxyalkyl, lower cycloalkyl, lower aryl, lower aralkyl, lower alkaryl, lower alkaralkyl, lower alkoxyaralkyl, and lower haloaralkyl, and the pharmacologically acceptable acid addition

salts thereof, said retinoid and said compound being applied in amounts which are effective to increase the rate of hair growth.

13. A method for treating alopecia caused by a shortening of the anagen (growing) phase of the hair cycle, which comprises topically applying to the scalp a minoxidil compound and a retinoid, said compound and said retinoid being applied in amounts which are effective to stimulate hair follicles of said scalp to produce hair growth therefrom.

14. A method for treating alopecia caused by a shortening of the anagen (growing) phase of the hair cycle, which comprises topically applying to the scalp a minoxidil compound and a retinoid, said compound and said retinoid being applied in amounts which are effective to prolong the anagen phase of the hair cycle.

15. A method for treating alopecia caused by a shortening of the anagen (growing) phase of the hair cycle, which comprises topically applying to the scalp a minoxidil compound and a retinoid, said compound and said retinoid being applied in amounts which are effective to convert vellus hair to growth as terminal hair.

16. A method of retarding shedding in fur bearing animals comprising topical administration to the animal of an effective amount of a retinoid and a minoxidil compound.

17. A method of retarding molting in birds comprising topical administration to the bird of an effective amount of a retinoid and a minoxidil compound.

18. In a method for treating alopecia caused by a shortening of the anagen (growing) phase of the hair cycle which comprises topically applying to the scalp an effective amount of a minoxidil compound, the improvement consisting of said topical application including a retinoid in an amount which is effective to increase the rate of hair growth.

19. A method for treating alopecia caused by a shortening of the anagen (growing) phase of the hair cycle, which comprises topically applying to the scalp a minoxidil compound and a retinoid, said compound and said retinoid being applied in amounts which are effective to increase the rate of hair growth.

20. A method according to claim 19 wherein said retinoid is Vitamin A acid.

21. A method according to claim 19 wherein said compound is minoxidil (2,4-diamino-6-piperidinopyrimidine-3-oxide).

22. A method according to claim 21 wherein said retinoid is Vitamin A acid.

23. A method according to claim 19 wherein said compound and said retinoid are applied in combination with a pharmaceutically acceptable vehicle.

24. A method according to claim 23 wherein said vehicle comprises a mixture of ethanol and propylene glycol.

25. The method of claim 19 wherein said retinoid is selected from the group consisting of all-trans retinoic acid, all-trans retinaldehyde, all-trans retinoyl acetate, and pharmaceutically acceptable salts, ethers, amides or esters thereof.

26. The method of claim 19 wherein the retinoid is an isomer of Vitamin A acid selected from the group consisting of 13-cis; 9,13-dicis; 9-cis; 11-cis; or 7,8-dehydro retinoic acid; Vitamin A_2 acid; α-Vitamin A acid; γ-Vitamin A acid; 5,6-epoxy Vitamin A acid; dehydrovitamin A acid; anhydro Vitamin A acid; and pharmaceutically acceptable salts of said isomer.

27. The method of claim 23 wherein said combination further comprises Vitamin D_3 or a Vitamin D_3 derivative selected from the group consisting of 1-hydroxycholecalciferol; 1,25-dihydroxycholecalciferol; and 1,24-dihydroxycholecalciferol.

28. The method of claim 23 wherein said combination further comprises a hormone selected from the group consisting of estrogens and progesterones.

29. The method of claim 23 wherein said combination further comprises an antiandrogen selected from the group consisting of cyproterone acetate, spironolactone, secosteroids, flutamides, cyoctol, and decahydro-7H-benz(E)-inden-7-ones.

30. A method for treating alopecia caused by a shortening of the anagen phase of the hair cycle which comprises topically applying to the scalp minoxidil and all-trans retinoic acid in amounts which are effective to increase the rate of hair growth.

* * * * *

(copy file #1

L4 ANSWER 8 OF 145 CAPLUS COPYRIGHT 2001 ACS
AN 1998:223670 CAPLUS
DN 128:286284
TI Transdermal delivery and accumulation of indomethacin in subcutaneous tissues in rats
AU Mikulak, Stephen A.; Vangsness, C. Thomas; Nimni, Marcel E.
CS Department of Surgery and Orthopaedics, School of Medicine, University of Southern California, Los Angeles, CA, 90033, USA
SO J. Pharm. Pharmacol. (1998), 50(2), 153-158
CODEN: JPPMAB; ISSN: 0022-3573
PB Royal Pharmaceutical Society of Great Britain
DT Journal
LA English
CC 63-5 (Pharmaceuticals)
Section cross-reference(s): 1
AB Oral non-steroidal anti-inflammatory drugs (NSAIDs) are effective pharmacotherapy for a wide variety of painful, inflammatory disorders. Development of an efficient means of topical administration of NSAIDs could increase local soft-tissue and joint concns. while reducing systemic distribution of the drug, thereby reducing side-effects. We studied the effects of a novel topical **penetration enhancer** for lipophilic compds., a trans-phase delivery system (TPDS), a soln. of benzyl alc., isopropanol and acetone, on the distribution of indomethacin in various tissues locally and remote from the site of application. We compared the TPDS with a 50:50 (vol./vol.) mixt. of **propylene glycol** and **ethanol**, a commonly used **penetration enhancer**, and with oral administration. The TPDS was significantly superior to the other approaches at achieving high local-tissue concns. in the vicinity of the site of application. In addn., comparison of these 2 carrier systems seems to clarify the different aq. and hydrophobic pathways of drug penetration which emerge from various exptl. findings and theor. considerations. This non-aq. solvent system, and benzyl alc. in particular, because of its unique physicochem. and solvating characteristics, might be able to deliver therapeutic levels of indomethacin to tissues close to the site of application in a safer and more effective manner than presently accepted forms of delivery.
ST transdermal delivery indomethacin subcutaneous tissue
IT Connective tissue
(s.c.; transdermal delivery and accumulation of indomethacin in s.c. tissues)
IT Drug bioavailability
Kidney
Liver
Transdermal drug delivery systems
(transdermal delivery and accumulation of indomethacin in s.c. tissues)
IT 53-86-1, Indomethacin
RL: BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(transdermal delivery and accumulation of indomethacin in s.c. tissues)
IT 67-63-0, Isopropanol, biological studies 67-64-1, Acetone, biological studies 100-51-6, Benzyl alcohol, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(transdermal delivery and accumulation of indomethacin in s.c. tissues)

US005482965A

United States Patent [19]

Rajadhyaksha

[11] Patent Number: 5,482,965

[45] Date of Patent: Jan. 9, 1996

[54] **COMPOSITIONS AND METHOD COMPRISING AMINOALCOHOL DERIVATIVES AS MEMBRANE PENETRATION ENHANCERS FOR PHYSIOLOGICAL ACTIVE AGENTS**

[76] Inventor: **Vithal J. Rajadhyaksha**, 27436 Esqina, Mission Viejo, Calif. 92691

[21] Appl. No.: **115,772**

[22] Filed: **Sep. 3, 1993**

Related U.S. Application Data

[63] Continuation of Ser. No. 672,020, Mar. 19, 1991, abandoned.

[51] **Int. Cl.**[6] **A61K 31/27**; A61K 31/335; A61K 31/22; A61K 31/225; A61K 31/23; A61K 31/16

[52] **U.S. Cl.** **514/452**; 514/478; 514/479; 514/546; 514/547; 514/552; 514/625; 514/629; 514/946; 514/947

[58] **Field of Search** 514/847, 785, 514/478, 479, 452, 546, 547, 552, 625, 629, 946, 947

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,247,256	6/1941	Senkus	260/338
2,260,265	10/1941	Senkus	260/338
2,317,555	4/1943	Robinette	252/357
2,320,707	6/1943	Robinette	252/355
2,346,454	4/1944	Robinette	252/8.75
2,370,586	2/1945	Senkus	260/338
2,383,622	8/1945	Senkus	260/338
2,399,068	4/1946	Senkus	260/338
2,415,021	1/1947	Morey	260/338
2,485,987	10/1949	Senkus	167/33
2,527,078	10/1950	Tucker	252/117
4,950,688	8/1990	Bowsett et al.	514/847
5,149,860	9/1992	Zysman et al.	560/160
5,198,470	3/1993	Zysman et al.	514/785

FOREIGN PATENT DOCUMENTS

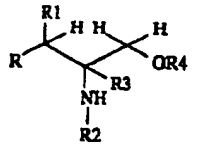
2652002	3/1991	France .
0268460	11/1987	WIPO .
WO88/04938	7/1988	WIPO .

Primary Examiner—T. J. Criares

Attorney, Agent, or Firm—Knobbe, Martens, Olson & Bear

[57] **ABSTRACT**

A method and compositions for enhancing absorption of topically administered physiologically active agents through the skin and mucous membranes of humans and animals in a transdermal device or formulation for local or systemic use, comprising a therapeutically effective amount of a pharmaceutically active agent and a non-toxic, effective amount of penetration enhancing agent of the formula I or a physiologically acceptable salt thereof:



wherein:

R^1, R^2, R^3 and R^4 are as defined herein.

26 Claims, No Drawings

Penetration Enhancer —

Hair treatment

COMPOSITIONS AND METHOD COMPRISING AMINOALCOHOL DERIVATIVES AS MEMBRANE PENETRATION ENHANCERS FOR PHYSIOLOGICAL ACTIVE AGENTS

This is a continuation of application Ser. No. 07/672,020, filed Mar. 19, 1991, now abandoned.

FIELD OF THE INVENTION

This invention relates to aminoalcohols and their derivatives as penetration-enhancers for pharmaceutical, agricultural and cosmetic agents.

BACKGROUND OF THE INVENTION

Many physiologically active agents are best applied topically to obtain desirable results. Topical application, in the form of creams, lotions, gels, solutions, etc., largely avoids side effects of the agents and permits high level concentrations of the agents.

Some therapeutic drugs may also be administered for systemic use through the skin or other body membranes including intranasal and intravaginal application of humans and other animals, utilizing a transdermal device or formulated in a suppository or aerosol spray. For some years, pharmaceutical researchers have sought an effective means of introducing drugs into the bloodstream by applying them to the unbroken skin. Among other advantages, such administration can provide a comfortable, convenient and safe way of giving many drugs now taken orally or infused into veins or injected intramuscularly.

Using skin as the portal for drug entry offers unique potential, because transdermal delivery permits close control over drug absorption. For example, it avoids factors that can cause unpredictable absorption from gastrointestinal tract, including changes in acidity, motility, and food content. It also avoids initial metabolism of the drug by the liver known as the first pass effect. Thus, controlled drug entry through skin can achieve a high degree of control over blood concentrations of drug.

Close control over drug concentration in blood can translate readily into safer and more comfortable treatment. When a drug's adverse effects occur at higher concentrations than its beneficial ones, rate control can maintain the concentration that evoke only—or principally the drug's desired actions. This ability to lessen undesired drug actions can greatly reduce the toxicity hazards that now restrict or prevent the use of many valuable agents.

Transdermal delivery particularly benefits patients with chronic disease. Many such patients have difficulty following regimens requiring several doses daily of medications that repeatedly cause unpleasant symptoms. They find the same drugs much more acceptable when administered in transdermal system that require application infrequently—in some cases, only once or twice weekly and reduce adverse effects.

Transdermal delivery is feasible for drugs effective in amounts that can pass through the skin area and that are substantially free of localized irritating or allergic effects. While these limitations may exclude some agents, many others remain eligible for transdermal delivery. Moreover, their numbers will expand as pharmaceutical agents of greater potency are developed. Particularly suitable for transdermal delivery are potent drugs with only a narrow

spread between their toxic and safe blood concentrations, those having gastrointestinal absorption problems, those susceptible to a higher first pass liver metabolism or those requiring frequent dosing in oral or injectable form.

Transdermal therapy permits much wider use of natural substances such as hormones. Often the survival times of these substances in the body are so short that they would have to be taken many times daily in ordinary dosage forms. Continuous transdermal delivery provides a practical way of giving them, and one that can mimic the body's own patterns of secretion.

At present, controlled transdermal therapy appears feasible for many drugs used for a wide variety of ailments including, but not limited to, circulatory problems, hormone deficiency, respiratory ailments, and pain relief.

Percutaneous administration can have the advantage of permitting continuous administration of drug to the circulation over prolonged periods of time to obtain a uniform delivery rate and blood level of drug. Commencement and termination of drug therapy are initiated by the application and removal of the dosing devices from the skin. Uncertainties of administration through the gastrointestinal tract and the inconvenience of administration by injection are eliminated. Since a high concentration of drug never enters the body, problems of pulse entry are overcome and metabolic half-life is not a factor of controlling importance.

The greatest problems in applying physiologically active agents topically or transdermally is that the skin is an effective barrier to penetration. The epidermis of the skin has an exterior layer of dead cells called the stratum corneum which is tightly compacted and oily and which provides an effective barrier against gaseous, solid or liquid chemical agents, whether used alone or in water or in oil solutions. If a physiologically active agent penetrates the stratum corneum, it can readily pass through the basal layer of the epidermis and into the dermis.

Although the effectiveness of the stratum corneum as a barrier provides great protection, it also frustrates efforts to apply beneficial agents directly to local areas of the body. The inability of physiologically active agents to penetrate the stratum corneum prevents their effective use of treating such conditions as inflammation, acne, psoriasis, herpes labialis, herpes genitalis, eczema, infections caused by fungi, viruses and other microorganisms, or or, her disorders or conditions of the skin or mucous membranes or of conditions beneath the exterior surface of the skin or mucous membranes. The stratum corneum also prevents the skin from absorbing and retaining cosmetic-type materials such as sunscreens, perfumes, mosquito repellents and the like.

Physiologically active agents may be applied to the locally affected parts of the body in the form of a solution, cream, lotion or gel utilizing the vehicle system described herein. These agents may also be delivered for systemic use utilizing the vehicle system in a transdermal patch. Vehicles such as USP cold cream, ethanol and various ointments, oils, solvents and emulsions have been used heretofore to apply physiologically active ingredients locally. Most such vehicles are not effective to carry significant amounts of physiologically active agents into and through the skin. One such vehicle is dimethyl sulfoxide, which is described in U.S. Pat. No. 3,551,554.

My previous inventions disclosed in U.S. Patent Nos. 3,989,816; 3,991,203; 4,122,170; 4,316,893; 4,405,616; 4,415,563; 4,423,040; 4,424,210; 4,444,762; 4,837,026 and 4,876,249 describe a method for enhancing the topical or transdermal administration of physiologically active agents

by combining such an agent with an effective amount of a penetration enhancer and applying the combination to skin or other body membranes of humans or animals, in the form of solution, cream, gel, lotion, or a transdermal device.

My related U.S. Patent Nos. 4,461,638 and 4,762,549 describe a method for enhancing delivery of plant nutrients and plant growth regulators, and my U.S. Pat. No. 4,525,199 describes an improved method of pest control by enhancing pesticide permeation.

My related U.S. application, Ser. No. 218,316, filed on Jul. 12, 1988, describes a method for enhancing topical and transdermal administration of physiologically active agents with membrane penetration enhancers selected from oxazolidone and related heterocyclic compounds.

My related U.S. application Set. No. 07/348,387, filed on May 8, 1989 describes a method for enhancing topical and transdermal administration of physiologically active agents with yet another series of membrane penetration enhancers.

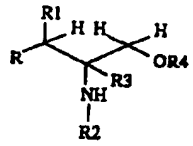
My related U.S. applications Ser. No. 07/393,584, filed on Aug. 11, 1989 and Set. No. 07/451,124, filed on Dec. 15, 1989, C.I.P.s of U.S. patent application Ser. No. 002,387, filed on Jan. 12, 1987, now U.S. Pat. No. 4,876,249, describe a method for enhancing topical and transdermal administration of physiologically active agents with membrane penetration enhancers selected from heterocyclic compounds containing two heteroatoms.

Penetration enhancers for enhancing systemic administration of therapeutic agents transdermally disclosed in the art include dodecyl pyrrolidone, dimethyl lauramide, dimethyl sulfoxide, decyl methyl sulfoxide, ethanol, 1-dodecylhexahydro-2H-azepin-2-one, 1-dodecanoyl hexamethyleneimine, 2-nonyl-1,3-dioxolane, fatty acids and their esters, sucrose esters etc. These agents may be used prior to or concurrently with administration of the active agent, see, e.g., U.S. Pat. Nos. 4,031,894; 3,996,934 and 3,921,636.

SUMMARY OF THE INVENTION

One of the main function of the epidermis is the production of a cohesive, relatively impermeable outer sheath. It has been known that from the time an epidermal cell leaves the basal layer to the time it is desquamated, the cell lipids change both qualitatively and quantitatively. A phospholipid is the most abundant lipid class in basal cell, whereas half of the lipid in a desquamated cell consists of ceramide. The lipid content of desquamated stratum corneum cell is approximately six time that of basal cell. The change in lipid composition of a cell undergoing cornification results mainly from de nuvo synthesis of cholesterol, fatty acid and ceramide.

This invention relates to penetranion enhancers closely related to the constituents of the epidermal outer sheath and therefore interact with it without irreversible disruption of the barrier. Moreover, these enhancers posseas an advantage that they are expected to yield nontoxic, pharmacologically inert metabolites after passage through the skin and the systemic circulation. The invention further relates to compositions for carrying physiologically active agents through body membranes such as skin and mucosa for retaining these agents in the body tissues and further relates to a method of administering systemically and locally active agents through the skin or other body membranes of humans and animals, utilizing a transdermal device or formulation, containing an effective, non-toxic amount of a membranae penetration enhancer having the structural formula I:



wherein:

R is selected from H, and an aliphatic hydrocarbon group with from about i to about 20 carbon atoms, optionally containing a heteroatom in the hydrocarbon chain;

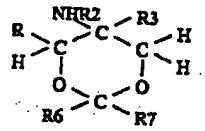
R1 is selected from H, OH or O-CO-RS, where R5 is an aliphatic hydrocarbon group with from about 1 to about 18 carbon atoms;

R2 is selected from H, a lower aliphatic hydrocarbon group, acyl, hydroxyacyl or alkoyloxyacyl group with up to about 40 carbon atoms;

R3 is selected from H, an aliphatic hydrocarbon group, with up to about 16 carbon atoms unsubstituted or substituted with hydroxy, acyloxy or alkylthio or an aryl or aralkyl group; and

R4 is H or an acyl group with from about 1 to about 18 carbon atoms; or

when R1 is OH_1R1and R4 are combined to form compounds having a 1,3 -dioxane ring,



wherein, R6 and R7 are selected from H, an aliphatic hydrocarbon group unsubstituted or substituted with hydroxy, acyloxy, or carboalkoxy, or an aryl group, or they may combine to form a carbonyl group,

or a physiologically acceptable salt thereof.

It is understood that the aliphatic hydrocarbon groups in the substituents R–R7 may be straight or branched and saturated or unsaturated, such as straight or branched chained alkyl, alkenyl or alkinyl groups. In the substituents where the hydrocarbon group may contain a heteroatom (R), this heteroatom usually is S or O.

It will be readily appreciated by those skilled in the art that certain compounds represented by formula I may exhibit optical and geometric isomerism. However, where no designation of isomers is specified with respect to the compounds of this invention, it is to be understood that all possible stereoisomers and geometric isomers (E and Z), and racemic and optically active compounds are included.

It will also be readily appreciated by those skilled in the art that certain of the compounds described in the disclosure may form salts with carboxylic and mineral acids and it is understood that all such salts, in particular the physiologically acceptable salts, are included in the invention.

In one preferred embodiment of I, R is an alkyl group with from 1 to 20 carbon atoms, R1 and R3 are H, R2 is an acyl group with from 1 to 30 carbon atoms and R4 is an acyl group with from 1 to 18 carbon atoms.

In another preferred embodiment of I, R1 is -O-CO-R5, wherein R5 is an alkyl group with from 1 to 18 carbon atoms and R, R2, R3 and R4 are as defined above.

Yet in another preferred embodiment of I, R1 is OH, R2 is H or acyl, R3 and R4 are H and R1 and R4 are combined to form a 1,3-dioxane ring and R, R6 and R7 are as defined above.

Yet in another preferred embodiment of I, R1 is OH, R2 is H or acyl, R3 is alkyl, aryl, aralkyl, hydroxyalkyl, acyloxyalkyl or alkylthioalkyl, R and R4 are H and R1 and R4 are combined to form a 1,3-dioxane ring, wherein R6 and R7 are as defined above.

It has been found that the physiologically active agents are carried through body membranes by the claimed penetration enhancers and are retained in the body tissue when applied topically in form of a cream, gel, or lotion or absorbed systemically when applied in the form of a transdermal device or formulation, for example, as a transdermal patch, a rectal or vagina suppository, as a nasal spray or when incorporated in a vaginal sponge or tampon.

This invention also relates to the problems such as skin irritation and skin sensitization that are commonly associated with conventional penetration enhancers found in the prior art. Since the compounds of this invention are structurally closely related to the ceramides, the lipids primarily present in the top layers of the skin, it is believed that skin irritation and skin sensitization can be avoided significantly with the use of these compounds as enhancers in the therapeutic compositions.

The invention further relates the penetration enhancers themselves and their method of making.

DETAILED DESCRIPTION OF THE INVENTION

Typical examples of compounds included in the foregoing formula I of this invention are the following:

1) 2-Ethanoylaminododecyl ethanoate

2) 2-Octanoylaminododecyl octanoate

3) 2-Octadec-9-enoylaminododecyl octadec-9-enoate

4) 2-Octadec-9-enoylaminododecyl ethanoate

5) 2-Octadecanoylaminooctadec-4-enyl-1,3-diethanoate

6) 2-Ethanoylaminooctadec-4-enyl 1,3-diethanoate

7) 2-Ethanoylaminooctadecyl 1,3-diethanoate

8) 5-Amino-2,2-dimethyl-4-(pentadec-1-enyl)-1,3-dioxane

9) 5-Amino-2,2-dimethyl-4-pentadecyl-1,3-dioxane

10) 5-Amino-4-(pentadec-1-enyl)-1,3-dioxan-2-one

11) 5-amino-4-dodecyl-1,3-dioxan-2-one

12) 4-Dodecyl-5-ethanoylamino-1,3-dioxan-2-one

13) 2-Ethanoylaminododecyl octadec-9-enoate

14) 2-Ethanoylamino-3-octadecyloxypropyl ethanoate

15) 5-Amino-2,2-dimethyl-4-(2,6,10,14-tetramethylpentadecyl)-1,3-dioxane

16) 5-Amino-2,2-dimethyl-4-(2,6-dimethyl-5-heptenyl)-1,3-dioxane

17) 5-amino-5-ethyl-2-undecyl-1,3-dioxane

18) 5-amino-2,2-dimethyl-5-undecyl-1,3-dioxane

19) 2,2-Dimethyl-5-dodecanoylamino-5-ethyl-1,3-dioxane

20) 5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane

21) 5-Amino-5-hydroxymethyl-2-(3-heptyl)-1,3-dioxane

22) 5-Amino-5-ethyl-2-carbobutoxyethyl-2-methyl-1,3-dioxane

23) 5-Dodecanoylamino-5-methyl-1,3-dioxan-2-one

24) 5-Amino-5-undecyl-1,3-dioxan-2-one.

The following compounds, encompassed by general formula I of this invention are known in the literature.

The 4E,2S,3R isomer of compound 6 is the triacetyl derivative of naturally occurring D-erythro-Sphingosine and has been synthesized by Findeis and Whitesides, J. Org. Chem. 52, 2838 (1987); Julina et. al. Helv. Chim. Acta 69, 368 (1986) and references cited therein; Schmidt and Zimmermann, Tet. Lett. 27, 481 (1986). The 2-octadecanoylamino 2S,3R 1,3-diol derivative of compound 5, a ceramide, has been synthesized by Julina et. al., loc. cit. Compound 7 is the dihydro derivative of compound 6 and the 2S,3R isomer has been prepared by Roush and Adam, J. Org. Chem. 50, 3752 (1985). The E isomer of the 4R,5S stereoisomers of compounds 8 and its corresponding 1-heptadecenyl analog have been synthesized, Hasegawa and Kiso, JPN. Kokai Tokyo Koho JP 62,207,247 [87,207,247], 11 Sep 1987, C.A., 108:P167212 (1988), Hino et. al. J. Chem. Soc. Perkin Trans. I, 1687 (1986) and both E and Z isomers have been prepared by Kiso et. al., Carbohydr. Res. 158, 101 (1986) and J. Carbohydr. Chem. 5, 335 (1986). 4R,5S isomer of Compound 9 have been prepared by Nakagawa et. al., Tet. Lett. 6281 (1987) during the synthesis of Cerebroside $B1_b$. and Saitoh et. al. Bull. Chem. Soc. Japan, 54, 488 (1981), who also prepared the 4-[(Z)-3pentadecenyl] analog of Compound 8 during the total synthesis of two prosopis alkaloids. Compounds 15 and 16 have been prepared by Umemura and Mori, Agric. Biol. Chem., 46, 1797 (1982) as intermediates in the synthesis of spinghosine analogs. Compound 17 and related 5-amino-1,3-dioxanes have been prepared by Senkus, J. Amer. Chem. Soc. 63, 2635 (1941), ibid., 65, 1656 (1943) and U.S. Pat. Nos. 2,247,256, 2,260,265, 2,370,586, 2,383,622, 2,399,068 and evaluated as coating compounds, as intermediates for the preparation of insecticides and surface active agents and as insecticides, U.S. Pat. No. 2,485,987 and by CIBA Ltd., Fr.1,457,767, as intermediates in the preparation of isonitriles useful as insecticides, acaricides, ovicides, herbicides, fungicides, bactericides, and molluscicides. Robinette, U.S. Pat. Nos. 2,317,555, 2,320,707 and 2,346,454, has studied the 5-amino-1,3-dioxanes as wetting, penetrating & cleansing agents for various textile and leather treatments. The 2-unsubstituted analog of Compound 19 has been utilized by Tucker, U.S. Patent No. 2,527,078, as an ingredient in detergent mixture for inhibiting the precipitation of lime soaps. Compound 20, 21 and analogs have been prepared and investigated by Senkus for insecticidal properties. Compound 22 has been prepared by Morey, U.S. Pat. No. 2,415,021. Aliphatic substituted 1,3-dioxacycloalkanes, without the prerequisite amino or substituted amino functionality of this invention, have been disclosed as skin penetration enhancers by Samour and Daskalakis, Eur. Pat. Appl. EP 268,460, 25 May 1988 and particularly, 2-nonyl-1,3-dioxolane, Proceed. Intern. Symp. Control Rel. Bioact. Mater. 17, 415 (1990) and references cited therein.

To my knowledge the other compounds are novel.

The use of the compounds of the present invention as penetration enhancers in drug delivery is, however, novel and not predictable from the prior art.

The aminoalcohol derivatives covered by the general formula I may be prepared by any of the processes known in the literature, and are hereby incorporated by reference. For example, Ohashi et. al., Tet. Lett. 29, 1185 (1988); Findeis and Whitesides, J. Org. Chem. 52, 2838 (1987); Nakagawa et. al., Tet. Lett. 6281 (1987); Hino et. al., J. Chem. Soc. Perkin Trans. I, 1687 (1986); Koike et. al., Carbohydr. res. 158, 113 (1986), Kiso et. al., Carbohydr. Res. 158, 101 (1986) and J. Carbohydr. Chem. 5, 335 (1986); Schmidt and Zimmermann, Tet. Lett. 481 (1986); Julina et. al. Helv. Chim. Acta 69, 368 (1986); Roush and Adam, J. Org. Chem. 50, 3752 (1985); Bernet and Vasella, Tet. Lett. 24, 5491 (1983); Chandrakumar and Hajdu, J. Org.

Chem. 48, 1197 (1983); Garigipati and Weinreb, J. Amer. Chem. Soc. 105, 4499 (1983); Schmidt and Klaeger, Angew. Chem. Suppl. 393 (1982) and Angew. Chem. Int. Ed. 21, 982 (1982); Umemura and Mori, Agric. Biol. Chem. 46, 1797 (1982); Saitoh et. al., Bull. Chem. Soc. Japan 54, 488 (1981); Newman, J. Amer. Chem. Soc.95, 4098 (1973) and Shapiro et. al., J. Amer. Chem. Soc. 80, 1194 (1958). In addition, the acetal and ketal derivatives of 5-amino-1,3-dioxane can be prepared from the nitro alcohols according to the methods of Senkus mentioned earlier and the corresponding 2-oxo derivatives by processes known for carbonyl group insertion such as those outlined in my pending U.S. application Ser. No. 218,316, filed on Jul. 12, 1988, followed by hydrogenation of the nitro group. 5-Acylamino-1,3-dioxanes can be easily prepared by acylation of the 5=amino compounds with an appropriate carboxylic acid derivative according to the well established methods in the literature. 5-Amino-1,3-dioxanes with other substituents in 2-position can be prepared by the treatment of the said nitro alcohols with compounds containing a carbonyl group and the desired functionality, for example, with butyl levulinate as outlined by Morey. Other amino alcohols can be prepared as outlined in my pending U.S. application Ser. No. 218,316, filed on Jul. 12, 1988 and derivatized to compounds of formula I.

The compounds of the present invention may be used as penetration enhancers in the same manner as described in my U.S. Pat. Nos. 3,989,816; 3,991,203; 4,415,563; 4,122,170; 4,316,893; 4,405,616; 4,415,563; 4,423,040; 4,424,210; 4,444,762; 4,837,026 4,876,249 and U.S. applications Ser. No. 218,316, filed on Jul. 12, 1988; Ser. No. 07/348,387 filed May 8, 1989; Ser. No. 07/393,584, filed Aug. 11, 1989, and Ser. No. 07/451,124, filed on Dec. 15, 1989, which are hereby incorporated by reference.

The compounds of the present invention are useful as penetration enhancers for a wide range of physiologically active agents and the compositions disclosed herein are useful for topical and transdermal therapeutic application of these agents. Typically systemically active agents which may be delivered transdermally are therapeutic agents which are sufficiently potent such that they can be delivered through the skin or other membranes to the bloodstream in sufficient quantities to produce the desired therapeutic effect. In general this includes agents in all of the major therapeutic areas including, but not limited to, anti-infectives, such as antibiotics and antiviral agents, analgesics and analgesics combinations, anorexics, anthelmintics, antiarthritics, antiasthma agents, anticonvul sants, antidepressants, antidiabetic agents, antidiarrheals, antihistamines, anti-inflammatory agents, antimigraine preparations, antimotion sickness, antinauseants, antineoplastics, antiparkinsonism drugs, antipruritics, antipsychotics, antipyretics, antispasmodics, including gastrointestinal and urinary; anticholinergics, sympathomimetics, xanthine derivatives, cardiovascular preparations including calcium channel blockers, beta-blockers, antiarrhythmics, antihypertensives, diuretics, vasodilators including general, coronary, peripheral and cerebral; central nervous system stimulants, cough and cold preparations, decongestants, diagnostics, hormones, hypnotics, immunosuppressives, muscle relaxants, parasympatholytics, parasympathomimetics, sedatives, tranquilizers and antiosteoporos is agents.

The subject compositions are also useful for topical application of many physiologically active agents in combination with the compounds of this invention.

Fungistatic and fungicidal agents such as, for example, thiabendazole, chloroxine, amphotericin, candicidin, fungimycin, nystatin, chlordantoin, clotrimazole, miconazole and related imidazole antifungal agents, pyrrolnitrin, salicylic acid, fezatione, ticlatone, tolnaftate, triacetin and zinc and sodium pyrithione may be combined with the compounds described herein and topically applied to affected areas of the skin. For example, fungistatic or fungicidal agents so applied are carried through the stratum corneum, and thereby successfully treat fungus-caused skin problems. These agents, thus applied, not only penetrate more quickly, but additionally enter the animal tissue in high concentrations and are retained for substantially longer time periods whereby a far more successful treatment is effected.

For example, the subject composition may also be employed in the treatment of fungus infections on the skin caused by candida and dermatophytes which cause athletes foot or ringworm, by incorporating thiabendazole or similar antifungal agents with one of the enhancers and applying it to the affected area.

The subject compositions are also useful in treating skin problems, such as for example, those associated with the herpes viruses, which may be treated with a cream of iododeoxyuridine or acyclovir in combination with one of the enhancers, or such problems as warts which may be treated with agents such as podophylline combined with one of the enhancers. Skin problems such as psoriasis may be treated by topical application of a conventional topical steroid formulated with one of the enhancers or by treatment with methotrexate incorporated with one of the enhancers of this invention. Scalp conditions such as alopecia areata may be treated more effectively by applying agents such as minoxidil in combination with one of the enhancers of this invention directly to the scalp.

The subject compositions are also useful for treating mild eczema, for example, by applying a formulation of Fluocinolone acetonide or its derivatives; hydrocortisone or triamcinolone acetonide incorporated with one of the enhancers to the affected area.

Examples of other physiologically active steroids which may be used with the enhancers include corticosteroids such as, for example, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorasone diacetate, flurandrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and its esters, chloroprednisone, clocorelone, descinolone, desonide, dexamethasone, dichlorisone, difluprednate, flucloronide, flumethasone, flunisolide, fluocinonide, flucortolone, fluoromethalone, fluperolone, fluprednisolone, meprednisone, methylmeprednisolone, paramethasone, prednisolone and prednisone.

The subject compositions are also useful in antibacterial chemotherapy, e.g. in the treatment of skin conditions involving pathogenic bacteria. Typical antibacterial agents which may be used in this invention include sul fonamides, penicillins, cephalosporins, erythromycins, lincomycins, vancomycins, tetracyclines, chloramphenicols, streptomycins, etc. Typical examples of the foregoing include erythromycin, erythromycin ethyl carbonate, erythromycin estolate, erythromycin glucepate, erythromycin ethylsuccinate, erythromycin lactobionate, lincomycin, clindamycin, tetracycline, chlortetracycline, demeclocycline, doxycycline, methacycline, oxtcetracycline, minocycline, etc.

The subject compositions are also useful in protecting ultra-sensitive skin or even normally sensitive skin from damage or discomfort due to sunburn. Thus, actinic dermatitis may be avoided by application of a sunscreen, such as PABA or its well known derivatives or benzophenones in combination with one of the enhancers, to skin surfaces that are to be exposed to the sun; and the protective agent will be

carried into the stratum corneum more successfully and will therefore be retained even when exposed to water or washing for a substantially longer period of time than when applied to the skin in conventional vehicles. This invention is particularly useful for ordinary suntan lotions used in activities involving swimming because the ultraviolet screening ingredients in the carriers are washed off the skin when it is immersed in water.

The subject compositions may also find use in treating scar tissue by applying agents which soften collagen, such as aminopropionitrile or penicillamine combined with one of the enhancers of this invention topically to the scar tissue.

Agents normally applied as eye drops, ear drops, or nose drops are more effective when combined with the enhancers of this invention.

Agents used in the diagnosis may be used more effectively when applied in combination with one of the enhancers of this invention. Patch tests to diagnose allergies may be effected promptly without scratching the skin or covering the area subjected to an allergen when the allergens are applied with one of the enhancers of this invention.

The subject compositions are also useful for topical application of cosmetic or esthetic agents. For example, compounds such as melanin-stimulating hormone (MSH) or dihydroxyacetone and the like are more effectively applied to the skin to simulate a suntan when they are used in combination with one of the enhancers of this invention. Depigmenting agents, such as hydroquinone, which bleach and lighten hyperpigmented skin are more effective when combined with one of the enhancers of this invention. Hair dyes also penetrate more completely and effectively when incorporated with enhancers of this invention. These enhancers are also useful in the compositions containing skin moisturizing agents.

The effectiveness of such topically applied materials as insect repellants or fragrances, such as perfumes and colognes, can be prolonged when such agents are applied in combination with the vehicles of this invention.

It is to be emphasized that the foregoing are simply examples of physiologically active agents including therapeutic and cosmetic agents having known effects for known conditions, which may be used more effectively for their known properties in accordance with this invention.

The term "physiologically active agent" is used herein to refer to a broad class of useful chemical and therapeutic agents including physiologically active steroids, antibiotics, anti-fungal agents, antibacterial agents, antineoplastic agents, allergens, antiinflammatory agents, antiemetics, antipruritic agents, antihistaminic agents, vasodilators, expectorants, analgesics, antiosteoporosis agents, sunscreen compounds, antiacne agents, collagen softening agents and other similar compounds. Cosmetic agents, hair and skin dyes, natural and synthetic hormones, perfumes, insect repellents, diagnostic agents and other such compounds may also be advantageously formulated with these penetration enhancers.

In addition, these membrane penetration enhancers may be used in transdermal applications in combination with ultrasound and iontophoresis.

Moreover, these penetration enhancers are useful in agriculture in the application of fertilizers, hormones, growth factors including micronutrients, insecticides, molluscicides, arachides, nematocides, rodenticides, herbicides, and other pesticides to plants, animals and pests. These penetration enhancers are also useful for penetration of micronutrients and chemical hybridization agents in seeds for enhanced plant growth. Of course, the appropriate dosage levels of all the physiologically active agents, without conjoint use of the penetration enhancing compounds of formula I, are known to those of ordinary skill in the art. These conventional dosage levels correspond to the upper range of dosage levels for compositions including a physiologically active agent and a compound of formula I as a penetration enhancer. However, because the delivery of the active agent is enhanced by compounds of the present invention, dosage levels significantly lower than conventional dosage levels may be used with success.

Systemically active agents are used in amounts calculated to achieve and maintain therapeutic blood levels in a human or other animal over the period of time desired. (The term "Animal" as used here encompasses humans as well as other animals, including particularly pets and other domestic animals.) These amounts vary with the potency of each systemically active substance, the amount required for the desired therapeutic or other effect, the rate of elimination or breakdown of the substance by the body once it has entered the bloodstream and the amount of penetration enhancer in the formulation. In accordance with conventional prudent formulating practices, a dosage near the lower end of the useful range of a particular agent is usually employed initially and the dosage increased or decreased as indicated from the observed response, as in the routine procedure of the physician.

The present invention contemplates compositions of compounds of formula I, together with physiologically active agents from 0.05% to 100% of conventional dosage levels. The amount of compound of Formula I which may be used in the present invention is an effective, non-toxic amount for enhancing percutaneous absorption. Generally, for topical use the amount ranges between 0.1 to about 10 and preferably about 0.1 to 5 percent by weight of the composition. For transdermal enhancement of systemic agents, the amount of penetration enhancer which may be used in the invention varies from about 1 to 100 percent although adequate enhancement of penetration is generally found to occur in the range of about 1 to 30 percent by weight of the formulation to be delivered. For transdermal use, the penetration enhancers disclosed herein may be used in combination with the active agent or may be used separately as a pre-treatment of the skin or other body membranes through which the active agent is intended to be delivered.

Dosage forms for application to the skin or other membranes of humans and animals include creams, lotions, gels, ointments, suppositories, sprays, aerosols, buccal and sublingual tablets and any one of a variety of transdermal devices for use in the continuous administration of systemically active drugs by absorption through the skin, oral mucosa or other membranes, see for example, one or more of U.S. Pat. Nos. 3,598,122; 3,598,123; 3,731,683; 3,742,951; 3,814,097; 3,921,636; 3,972,995; 3,993,072; 3,993,073; 3,996,934; 4,031,894; 4,060,084; 4,069,307; 4,201,211; 4,230,105; 4,292,299 and 4,292,303. U.S. Pat. No. 4,077,407 and the foregoing patents also disclose a variety of specific systemically active agents which may also be useful as in transdermal delivery, which disclosures are hereby incorporated herein by this reference.

The penetration enhancers of this invention may also be used in admixture with other penetration enhancers disclosed earlier and incorporated herein by reference.

Typical inert carriers which may be included in the foregoing dosage forms include conventional formulating materials, such as, for example, water, ethanol, 2-propanol, 1,2-propanediol, 1,3-butanediol, 2-octyldodecanol, 1,2,3,-propanetriol, oleyl alcohol, propanone, butanone, carboxylic

acids such as lauric, oleic and linoleic acid, carboxylic acid esters such as isopropyl myristate, diisopropyl adipate and glyceryl oleate, acyclic and cyclic amides including N-methyl pyrrolidone, urea, freons, PEG-200, PEG-400, Polyvinyl pyrrolidone, fragrances, gel producing materials such as "Carbopol", stearyl alcohol, stearic acid, spermaceti, sorbitan monooleate, sorbital, "polysorbates", "Tweens", methyl cellulose etc., antimicrobial agent/preservative compositions including parabens, benzyl alcohol, potassium sorbate, sorbic acid, or a mixture thereof and antioxidant such as BHA or BHT. The dosage form may include a corticosteroid, such as hydrocortisone, to prevent skin sensitization, a local anaesthetic, such as lidocaine or benzocaine to suppress local irritation.

The examples which follow illustrate the penetration enhancers and the compositions of the present invention. However, it is understood that the examples are intended only as illustrative and are not to be construed as in any way limiting to scope of this invention.

EXAMPLE 1

Preparation of 2-Ethanoylaminododecyl ethanoate

To a solution of 4.1 g of 2-aminododecanol, 5 g of triethylamine in 100 ml of dichloromethane was slowly added 3.2 ml of acetyl chloride. The reaction mixture was stirred for 3 hours and then quenched by pouring into ice. The aqueous solution was extracted with dichloromethane. The organic layer was washed with water, brine and then dried, filtered and concentrated to 5.7 g of a waxy solid. Recrystallization from ether/hexane gave 4.22 g (72.2%) of the desired amidoester as white crystals, m.p. 77°–79° C.

EXAMPLE 2

Preparation of 5-Amino-5-ethyl-2-carbobutoxyethyl-2-methyl-1,3-dioxane

7.46 g of 2-nitro-2-ethyl-1,3-propanediol, 8.61 g of butyl levulinate, 50 mg of p-toluenesulfonic acid in 50 ml of toluene was refluxed until no more water separated. The reaction mixture was cooled, washed with 2% sodium bicarbonate and water, dried and concentrated to give 13.65 g of 2-carbobutoxyethyl-2-methyl-5-nitro-5-ethyl-1,3-dioxane as a light yellow oil. This was dissolved in 50 ml of ethanol and hydrogenated over 1 g Raney Nickel catalyst at 60 under pressure. Distillation of the crude material at 160° C./3mm gave 11 g of the product.

EXAMPLE 3

Preparation of 5-Amino-5-ethyl-2- (3 -heptyl) -1,3-dioxane

Procedure of Example 2 was repeated with 6.41 g of 2-ethylhexanal in place of butyl levulinate to give 11.6 g of the 5-nitro-1,3-dioxane, which was reduced and distilled at 135°–137° C./10 mm to give 9.23 g of the product.

EXAMPLE 4

Preparation of 5-Amino-5-hydroxymethyl-2-(3-heptyl)-1,3-dioxane

Procedure of Example 2 was repeated with 6.41 g of 2-ethylhexanal and 7.56 g of 2-(hydroxymethyl)-2-nitro-1,3-propanediol to give 11 g of 5-nitro-5-hydroxymethyl-1,3-dioxane derivative, which was reduced and distilled at 175°–178° C. to give 8.7 g of the product.

EXAMPLE 5

Preparation of 5-Amino-5-ethyl-2-undecyl-1,3-dioxane

Procedure of Example 2 was repeated with 9.216 g of dodecanal in place of butyl levulinate to give 13.4 g of the 5-nitro-1,3-dioxane derivative. Hydrogenation followed by distillation of the crude liquid at 150° C./1 mm gave 10.9 g of the product.

EXAMPLE 6

Preparation of erythro-5-Amino-2,2-dimethyl-4-[(E)-pentadec-1-enyl]-1,3-dioxane

17.6 g of nitroethanol was added to a solution of 22 g of (E)-hexa-dec-2-enal in 160 ml of triethylamine under an inert atmosphere. The mixture was stirred and the reaction was followed by t.l.c. After 4 days the reaction mixture was concentrated and the residue was dissolved in dichloromethane. This was washed with ice-cold 5% HCl, water, dried and concentrated to give an orange oil. This was flash chromatographed (silica gel: hexane/ethyl acetate, 7:3) to give 21.2 g of a mixture of threo- and erythro-nitro diols. 20.9 g of the isomeric mixture, 500 ml of 2,2-dimethoxypropane and 100 mg of camphor-10-sulfonic acid was refluxed overnight under an inert atmosphere. The reaction mixture was cooled, concentrated and the residue was dissolved in dichloromethane. The organic solution was washed with bicarbonate solution, water and brine. It was dried and concentrated to give a mixture of acetonides which were dissolved in benzene and the solution was refluxed for 8 hours in presence of Merck silica gel-60. The mixture was filtered and the silica gel was washed with warm benzene. The filtrate was concentrated and the residue was chromatographed to give 16.7 g of erythro-nitro acetonide. To a suspension of 5 g of lithium aluminum hydride in 200 ml of THF was added dropwise a solution of 16.7 g of the erythro-nitro acetonide in 100 ml of THF at room temperature. The reaction mixture was stirred for 8 hours and then excess LAH was quenched with water. THF was removed under reduced pressure, the residue was diluted with ethyl acetate and the mixture was filtered. The organic layer was separated, washed with water, brine and dried. Concentration of the filtrate under reduced pressure gave 15.1 g of erythro-5-Amino-4-[(E)-pentadec-1-enyl]-1,3-dioxane as an oil.

EXAMPLE 7

Preparation of erythro-5-Amino-2,2-dimethyl-4-pentadecyl-1,3-dioxane

3 g of the material obtained under Example 6 was dissolved in 50 ml of methanol and hydrogenated over 100 mg of platinum oxide catalyst. Filtration and concentration gave 2.86 g of an oil.

EXAMPLE 8

Preparation of erythro and threo-5-Amino-2,2-dimethyl-4-(2,6-dimethyl-5-heptenyl)-1,3-dioxane

To a mixture of 11,565 g of racemic citronellal and 13.65 g of 2-nitroethanol was added 872 mg of KF and 1.21 g of tetra-n-butylammonium bromide in 75 ml of acetonitrile and the mixture was stirred at room temperature under an inert atmosphere. After 24 hours the reaction mixture was poured into ice-cold water and extracted with ether. The ether extract was washed with water and brine, dried and concentrated to give 15.6 g of isomeric mixture of 5,9-dimethyl-2-nitro-S-decene-1,3-diol.

A mixture of 14,715 g of the nitrodiol, 18.75 g of 2,2-dimethoxy-propane and 30 mg of p-toluenesulfonic acid in 150 ml of toluene was heated to reflux and water was removed by azeotropic distillation. The reaction mixture was cooled, diluted with ether and this was washed with water, brine, dried and concentrated in vacuo to give a yellow oil. The two isomers were separated by chromatography on Merck silica gel 60 and solution with benzene. 5.9 g of equatorial isomer was obtained first followed by 7.9 g of axial isomer, both as pale yellow oils.

To an ice-cold mixture of 4.5 g of the equatorial nitro isomer in 210 ml of ether and 13.5 ml of water was added freshly prepared amalgamated aluminum under stirring. The temperature of the reaction mixture was allowed to come to room temperature and then it was stirred for an additional 24 hours. The reaction mixture was filtered through celite and the filter cake was thoroughly washed with ether. The filtrate was concentrated to give an oil which was passed through neutral alumina to give 3 g of erythro isomer of 5-amino-2,2-dimethyl-4-(2,6-dimethyl-5-heptenyl)-1,3-dioxane as a colorless oil. 7.5 g of the axial nitro isomer was similarly reduced to give 4.99 g of the threo isomer as a colorless oil.

EXAMPLE 9

Preparation of 2-Octanoylaminododecyl octanoate

To a solution of 2 g of 2-aminododecanol, 3 g of triethylamine in 50 ml of dichloromethane is added 3.5 g of octanoyl chloride. The reaction mixture is stirred overnight and then quenched by pouring into ice. This is extracted with dichloromethane and the organic solution is washed with aqueous bicarbonate solution, water and brine. The organic phase is dried over magnesium sulfate, filtered and concentrated to give 3.8 g of the product.

EXAMPLE 10

Preparation of 2-Octadec-9-enoylamincdodecyl octadec-9-enoate

Example 9 is repeated under identical conditions with a solution of 2 g of 2-aminododecanol, 3 g of triethylamine in 50 ml of dichloromethane to which is added 6.3 g of oleoyl chloride. The reaction mixture is worked up as under Example 8 to give 5.1 g of product.

EXAMPLE 11

Preparation of 2-Ethanoylaminododecyl octadec-9-enoate

To a solution of 2.43 g of 2-ethanoylaminododecanol, 3 g of triethylamine in 50 ml of dichloromethane is added 3.2 g of oleoyl chloride. The reaction mixture is worked up as under Example 9 to give 4.2 g of product.

EXAMPLE 12

Preparation of 2,2-Dimethyl-5-dodecanoylamino-5-ethyl-1,3-dioxane

2,2-Dimethyl-5-amino-5-ethyl-1,3-dioxane is acylated with dodecanoic acid in methylene chloride in the presence of DCC and 1-hydroxybenzo-triazole. Filtration and concentration gives the product.

EXAMPLE 13

Preparation of 5-Dodecanoylamino-5-methyl-1,3-dioxan-2-one

A solution of 2-methyl-2-nitro-1,3-propanediol and ethylene carbonate is heated overnight. The reaction mixture is diluted with ethyl acetate and the solution is washed with water. The organic phase is dried and concentrated to obtain 5-methyl-5-nitro-1,3-dioxan-2-one. This is dissolved in methanol and hydrogenated under pressure to give the 5amino compound which is acylated with dodecanoyl chloride to give the product.

EXAMPLE 14

Preparation of 5-Amino-5-undecyl-1,3-dioxan-2-one

2-Nitro-2-undecyl-1,3-propanediol is treated under identical conditions according to the reaction sequence outlined under Example 13 to give the product.

EXAMPLE 15

The following analgesic gel is prepared:

	%
Carbopol 941	1.5
Diclofenac Na	1
2-Propanal	35
Diisopropanolamine	1.8
Diisopropyl adipate	5
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	2
Water	53.7

EXAMPLE 16

The following cream formulation is prepared:

	%
Isosorbide dinitrate	1.0
Glycerol monostearate	5.5
Polyoxyethylene stearate	4.5
C8–C18 fatty acid esters of a glycerol ethoxylated with about 7 moles of ethylene oxide	8
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	2
Sorbic acid	0.165
Ascorbyl palmitate	0.055
Citric acid	0.1
Na EDTA	0.014
Fragrance	0.05
Water	78.616

This formulation is effective in the treatment of angina.

EXAMPLE 17

The following skin moisturizing formulation is prepared:

	%
Pyrrolidonocarboxylic acid Na	1
Glycerine	4
Citric acid	0.03
Sodium citrate	0.05
Allantoin	0.1
Ethanol, 95%	9
Oleth-15	1
Linaleic acid	1
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	2
Sunscreen agent	0.1
Water	81.72

EXAMPLE 18

The following formulation for promoting hair growth is described.

	%
Minoxidil	2.0
Benzyl nicotinate	0.5
Ethanol	40.0
1,2-Propanediol	20.0
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	5.0
Ethyl oleate	5.0
Water	27.5

EXAMPLE 19

The following solution formulation is prepared.

	%
Griseofulvin	1
5-Amino-5-ethyl-2-(3-heptyl)1,3-dioxane	1.5
C12–C15 benzoate	5
Fragrance	0.1
Ethanol	92.4

This formulation is effective in the treatment of fungus infection.

EXAMPLE 20

The following depilatory gel is prepared.

	%
Poloxamer 407	15.0
Benzyl alcohol	6.0
Urea	6.5
alpha-Thioglycerol	6.5
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	5.0
Water	q.s. 100.0
Sodium hydroxide	q.s. to pH 12.5

EXAMPLE 21

The following cream formulation is prepared:

	%
Clindamycin Base	1.0
Stearyl alcohol, U.S.P.	12.0
Ethoxylated cholesterol	0.4
Synthetic spermaceti	7.5
Sorbitan monooleate	1.0
Polysorbate 80, U.S.P.	3.0
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	1.9
Sorbitol solution, U.S.P.	5.5
Sodium citrate	0.5
Chemoderm #844	0.2
Purified water	67.0

This formulation is effective in the treatment of ache.

EXAMPLE 22

The following solution formulations are prepared:

	A (%)	B (%)
Clindamycin base	—	1.0
Clindamycin phosphate acid	1.3	—
Sodium hydroxide	0.077	—
1M Hydrochloric acid	—	2.27
Disodium edentate.2H20	0.003	0.003
Fragrances	0.5	0.5
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	1.0	1.0
Purified water	20.0	17.73
Isopropanol	77.12	77.497

These solutions are effective for the treatment of ache in humans.

EXAMPLE 23

The following solution formulation is prepared:

	%
Neomycin sulfate	0.5
Lidocaine	0.5
Hydrocortisone	0.25
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	1.50
Propylene glycol	97.25

This solution is effective for the treatment of otitis in domestic animals.

EXAMPLE 24

The following sunscreen emulsion is prepared:

	%
PABA	2.0
Benzyl alcohol	0.5
5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane	2.0
Polyethylene glycol	9.0
Isopropyl lanolate	3.0
Lantrol	1.0
Acetylated lanolin	0.5
C12–C15 benzoate	5.0
Diisopropyl adipate	2.0
Cetyl alcohol	1.0
Veegum	1.0